REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-02-

0240

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
		01 Dec 98 to 30 Nov 01 FINAL

4. TITLE AND SUBTITLE
Pulsed Electric Field effects on Biological Cells

5. FUNDING NUMBERS
61102F
2301/EX

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Old Dominion Univ Rsch Foundation Inc
800 West 46th Street
PO Box 6369
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NE
801 North Randolph Street Rm 732
Arlington, VA 22203-1977

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
F49620-99-1-0069

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
APPROVAL FOR PUBLIC RELEASED; DISTRIBUTION UNLIMITED

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

A new type of field-cell interaction, "Intracellular Electromanipulation", by means of nonthermal, wideband electromagnetic radiation is being studied. It is based on capactive coupling to cell substructures, and has therfore the potential to affect transport processes across subcellular membrances. This was verified experimentally by applying electrical pulses of 60 nanosecond duration, with electric field amplitudes of 50 kV/cm to human eosinophils in vitro. Besides causing poration of intracellular membrances without disrupting the outer cell membrance, sub-microsecond pulses were found to induce apoptosis in cells.

14. SUBJECT TERMS			15. NUMBER OF PAGES
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

Final Report
on the Project

Pulsed Electric Field Effects on Biological Cells

Grant No: F49620-99-1-00069

December 1, 1998 to November 31, 2001

submitted to

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Abstract

A new type of field-cell interaction, "Intracellular Electromanipulation", by means of nonthermal, wideband electromagnetic radiation is being studied. It is based on capacitive coupling to cell substructures, and has therefore the potential to affect transport processes across subcellular membranes. This was verified experimentally by applying electrical pulses of 60 nanosecond duration, with electric field amplitudes of >50 kV/cm to human eosinophils in vitro. Besides causing poration of intracellular membranes without disrupting the outer cell membrane, sub-microsecond pulses were found to induce apoptosis in cells. This effect is being explored for cancer treatment. Experiments with sub-microsecond electric field exposure on a mouse model showed reduced tumor growth. In addition to wideband radiation effects, the response of cells to narrowband electromagnetic radiation in selected millimeter-wave (41 GHz – 43 GHz), and radio-wave (800 kHz to 850 KHz) ranges was studied at moderate power levels (mW/cm^2 to W/cm^2). The results of experiments in both wavelength ranges did not indicate any resonance effects. A third field of bioelectric research, bacterial decontamination using nanosecond pulsed elctric fields and pulsed corona discharges in water, has been initiated. First results indicate increased decontamination efficiency with cold plasmas in water compared to pulsed electric fields.

Summary

The program in Basic and Applied Bioelectrics at Old Dominion University and Eastern Virginia Medical School, which was initiated and supported by this grant contains a medical/ biological and an environmental component. Research efforts have

a) focused on the effect of high power, ultra-fast electrical pulses (wideband radiation) and that of moderate power, cw narrowband radiation on mammalian cells and tissues.
b) Experiments on the effect of pulsed electric fields on bacteria either by direct irradiation or through electrical discharges have been performed.

The bioelectric techniques which are based on the **ultrafast (nanosecond) pulse-cell interactions** are based on a predicted, and recently observed effect that ultrashort pulses (wideband electromagnetic radiation) permeate the outer membrane and affect cell substructures. One of the consequences of this effect is the electrically triggered programmed cell death: apoptosis. The research in this field has stimulated a strong interest in electro-bio-technology, as indicated by international attendance at the second conference on this topic, ElectroMed2001, which was chaired by one of the Co-PIs on this grant, Stephen Beebe. Other indicators for the interest of the scientific community in Bioelectrics is the large number of invited talks, among them a plenary talk at a major conference (PPPS2001). The enclosed review paper based on this talk contains a summary of the status in modeling and experimental research in this new field of pulsed electric field-cell interactions.

Besides using wideband radiation to affect cell structures and functions, the **response of narrowband electromagnetic radiation on cells** was explored. Since resonance-like biological effects of millimeter wave radiation at frequencies of approximately 42 GHz on the growth rate of *E. coli* and on DNA had been reported in the scientific literature, we have focused on this frequency range. The growth rate and the absorption spectrum of *E. coli*, irradiated by millimeter waves in the frequency range from 41 GHz to 43 GHz was measured, and the effect of this radiation on DNA was studied by measuring plasmid transformation efficiency. Both the growth rate variations with varying frequency and the variations in the result of the plasmid transformation efficiency experiments were found to be statistically insignificant. Resonance-like absorption features observed in the absorption spectrum of *E. coli* were identified as modes generated in the millimeter wave system, when the sample was inserted. The experimental results indicate that resonance effects are unlikely in this particular frequency range. The millimeter wave study was complemented by an experimental study of radio-frequency effects on gene expression. Effects on gene expression were determined by transient transfections of human cells with two different reporter plasmids. The results of this study showed that there were no effects in gene expression after exposure to 800, 835, and 847.74 MHz at electric field strengths of 0.83, 1.67, and 2.64 V/cm.

A third field of research supported by this grant is bacterial decontamination of liquids. We have focused on the **effect of ultrawideband pulses on bacteria**. These pulsed

electric field studies have recently been extended to cold plasma decontamination methods, where the plasma is generated in the liquid itself. Results with pulsed corona discharges in water have indicated a high efficiency of **bacterial decontamination by means of cold plasma in liquids**. The leading role of the research team at Old Dominion University in pulsed field bacterial decontamination is demonstrated by the invitation to write a review paper on this topic in the IEEE Transaction on Dielectrics and Insulation, and the appointment of the P.I. of this project as Chair of the Biodielectrics Committee of the IEEE Dielectrics and Electrical Insulation Society.

Achievements (manuscripts related to the particular achievements are listed in italics)

- **overview on status of "bioelectrics" research**

  Karl H. Schoenbach, Sunao Katsuki, Robert H. Stark, Stephen Beebe, and Stephen Buescher , "Bioelectrics – New Applications for Pulsed Power Technology," to appear in IEEE Trans. Plasma Science.

  Karl H. Schoenbach, Robert H. Stark, Stephen Beebe, and Stephen Buescher, "Bioelectrics – New Applications for Pulsed Power Technology," Proc. PPPS2001, Las Vegas, NV, June 2001.

- **concept of intracellular poration, and first demonstration of intracellular poration without permanent damage to outer cell membrane**

  Karl H. Schoenbach, "Deactivation of Microbial Pathogens with Intense, Ultrashort Electrical Pulses and High Power Microwave Radiation," Proc. 1998 ERDEC Scientific Conf. on Chemical and Biological Defense Research, Aberdeen Proving Ground, MD, November 1998, p. 583.

  Karl H. Schoenbach, Stephen J. Beebe, and E. Stephen Buescher, "Intracellular Effect of Ultrashort Pulses," J. Bioelectromagnetics 22, 440 (2001).

- **induction of apoptosis (programmed cell death) in mammalian cells**

  S.J. Beebe, P.M. Fox, L.J. Rec, K. Somers, R.H. Stark and K.H. Schoenbach "Nanosecond Pulsed Electric Field (nspef) Effects on Cells and Tissues: Apoptosis Induction and Tumor Growth Inhibition," to appear in IEEE Trans. Plasma Science.

  S.J. Beebe, P.M. Fox, L.J. Rec, K. Somers, R.H. Stark and K.H. Schoenbach "Nanosecond Pulsed Electric Field (nspef) Effects on Cells and Tissues: Apoptosis Induction and Tumor Growth Inhibition," Proc. PPPS2001, Las Vegas, NV, June 2001

  J. Deng, K.H. Schoenbach, E.S. Buescher, and S.J. Beebe, "From Electroporation to Apoptosis: the Effect of Intense, Submicrosecond Electrical Pulses on Cells," submitted to J. Biophysics

- **studies on the effect of microwave (41 GHz to 43 GHz) and radiowave (800 kHz to 850 kHz) irradiation on E. coli, DNA, and mammalian cells**

  G. Yu, E. Coln, K.H. Schoenbach, M. Gellerman, P. Fox, L. Rec, S. Beebe, and S. Liu, "A Study on Biological Effects of Low-Intensity Millimeter Waves," submitted to IEEE Trans. Plasma Science.

  Elizabeth A. Coln "A Study of Radiofrequency Effects on Gene Expression", M.S. Thesis, December 10, 2001(Old Dominion University, Department of Electrical and Computer Engineering)

- **modeling of the pore kinetics in cell membranes under the influence of ultrashort electrical pulses**

  R.P. Joshi and K.H. Schoenbach, "Electroporation Dynamics in Biological Cells Subjected to Ultrafast Electrical Pulses: A Numerical Simulation Study," Phys. Rev. E 62, 1025 (2000).

  R.P. Joshi, Q. Hu, R. Aly, K.H. Schoenbach, and H.P. Hjalmarson, "Self-Consistent Simulations of Electroporation Dynamics in Biological Cells Subjected to Ultrashort Electrical Pulses," Phys. Rev. E 64, (2001)

  R.P. Joshi, Q. Hu, K.H. Schoenbach, and H.P. Hjalmarson, "Theoretical Predictions of Electro-Mechanical Deformation of Cells Subjected to High Voltages for Membrane Electroporation," to appear in Phys. Rev. E.

- **studies on pulsed electric field effects on bacteria**

  Karl H. Schoenbach, Ravindra P. Joshi, Robert H. Stark, Frederick Dobbs, and Stephen J. Beebe, "Bacterial Decontamination of Liquids with Pulsed Electric Fields," ReviewPaper, IEEE Trans. on Dielectrics and Electrical Insulation 7, 637 (2000).

  R. E. Aly, R. P. Joshi, R. H. Stark, K. H. Schoenbac, and S. Beebe, "The Effect of Multiple, Microsecond Electrical Pulses on Bacteria," Proc. PPPS2001, Las Vegas, NV, June 2001.

- **bacterial decontamination of water using cold plasmas**

  A. Abou-Ghazala, S. Katsuki, K.H. Schoenbach, F.C. Dobbs, and K.R. Moreira, "Bacterial Decontamination of Water by Means of Pulsed Corona Discharges," submitted to IEEE Trans. Plasma Science.

  A. Abou-Ghazala, S. Katsuki, K. H. Schoenbach, F. C. Dobbs, and K. R. Moreira, "Bacterial Decontamination of Water by Means of Pulsed Corona Discharges," Proc. PPPS2001, Las Vegas, NV, June 2001.

- **development of pulsed power systems for bioelectrics**

  K.H. Schoenbach, R.H. Stark, J. Deng, R. El-Sayed Aly, S.J. Beebe, and E.S. Buescher, "Biological/Medical Pulsed Electric Field Treatments," Conf. Record, 2000 Twenty-Fourth Intern. Power Modulator Symposium, June 2000, Norfolk, VA, p. 42.

  J. Deng, R.H. Stark, and K.H. Schoenbach, "A Nanosecond Pulse Generator for Intracellular Electromanipulation," Conf. Record, 2000 Twenty-Fourth Intern. Power Modulator Symposium, June 2000, Norfolk, VA, p. 47.

- **organization of the Second International Symposium on Nonthermal Medical/Biological Treatments Using Electromagnetic Fields and Ionized Gases, "ElectroMed2001" in Portsmouth, VA, May 21 – 23, 2001**

  Symposium Record Abstracts

S. Beebe: Chairman

- **sixteen invited presentation on the topic of Bioelectrics, two of them plenary talks, and one of them a keynote address.**
  listed in the Bibliography section of this report

- **patent on "Bioelectrics"**
  Method and Apparatus for Intracellular Electromanipulation
  Patent No: 6,326,177
  Patent Date: December 4, 2001
  Inventors: Karl H. Schoenbach, Stephen J. Beebe, and E. Stephen Buescher

Publications, Presentations, and Patents

A. Journal Publications

1. R. P. Joshi, Q. Hu, K. H. Schoenbach, and S. J. Beebe, "Simulations of Electroporation Dynamics and Shape Deformations in Biological Cells Subjected to High Voltage Pulses," submitted to IEEE Trans. Plasma Science.

2. A. Abou-Ghazala, S. Katsuki, K.H. Schoenbach, F.C. Dobbs, and K.R. Moreira, "Bacterial Decontamination of Water by Means of Pulsed Corona Discharges," submitted to IEEE Trans. Plasma Science.

3. G. Yu, E. Coln, K.H. Schoenbach, M. Gellerman, P. Fox, L. Rec, S. J. Beebe, and S. Liu, "A Study on Biological Effects of Low-Intensity Millimeter Waves," submitted to IEEE Trans. Plasma Science.

4. J. Deng, K.H. Schoenbach, E.S. Buescher, and S.J. Beebe, "From Electroporation to Apoptosis: the Effect of Intense, Submicrosecond Electrical Pulses on Cells," submitted to J. Biophysics.

5. R.P. Joshi, Q. Hu, K.H. Schoenbach, and H.P. Hjalmarson, "Theoretical Predictions of Electro-Mechanical Deformation of Cells Subjected to High Voltages for Membrane Electroporation," to appear in Phys. Rev. E.

6. Karl H. Schoenbach, Sunao Katsuki, Robert H. Stark, Stephen Beebe, and Stephen Buescher , "Bioelectrics – New Applications for Pulsed Power Technology," to appear in IEEE Trans. Plasma Science.

7. S.J. Beebe, P.M. Fox, L.J. Rec, K. Somers, R.H. Stark and K.H. Schoenbach "Nanosecond Pulsed Electric Field (nspef) Effects on Cells and Tissues: Apoptosis Induction and Tumor Growth Inhibition," to appear in IEEE Trans. Plasma Science.

8. R.P. Joshi, Q. Hu, R. Aly, K.H. Schoenbach, and H.P. Hjalmarson, "Self-Consistent Simulations of Electroporation Dynamics in Biological Cells Subjected to Ultrashort Electrical Pulses," Phys. Rev. E 64 (2001).

9. Karl H. Schoenbach, Stephen J. Beebe, and E. Stephen Buescher, "Intracellular Effect of Ultrashort Pulses," J. Bioelectromagnetics 22, 440 (2001).

10. Karl H. Schoenbach, Ravindra P. Joshi, Robert H. Stark, Frederick Dobbs, and Stephen J. Beebe, "Bacterial Decontamination of Liquids with Pulsed Electric Fields," Review Paper, IEEE Trans. on Dielectrics and Electrical Insulation 7, 637 (2000).

11. R.P. Joshi and K.H. Schoenbach, "Electroporation Dynamics in Biological Cells Subjected to Ultrafast Electrical Pulses: A Numerical Simulation Study," Phys. Rev. E 62, 1025 (2000).

B. Publications in Conference Proceedings

1. Karl H. Schoenbach, Robert H. Stark, Stephen Beebe, and Stephen Buescher, "Bioelectrics – New Applications for Pulsed Power Technology," Proc. PPPS2001, Las Vegas, NV, June 2001

2. S.J. Beebe, P.M. Fox, L.J. Rec, K. Somers, R.H. Stark and K.H. Schoenbach "Nanosecond Pulsed Electric Field (nspef) Effects on Cells and Tissues: Apoptosis Induction and Tumor Growth Inhibition," Proc. PPPS2001, Las Vegas, NV, June 2001

3. A. Abou-Ghazala, S. Katsuki, K. H. Schoenbach, F. C. Dobbs, and K. R. Moreira, "Bacterial Decontamination of Water by Means of Pulsed Corona Discharges," Proc. PPPS2001, Las Vegas, NV, June 2001.

4. R. E. Aly, R. P. Joshi, R. H. Stark, and K. H. Schoenbach, "The Effect of Multiple, Microsecond Electrical Pulses on Bacteria," Proc. PPPS2001, Las Vegas, NV, June 2001

5. Invited: Karl H. Schoenbach, Jingdong Deng, Guofen Yu, Robert H. Stark, Stephen J. Beebe, and E. Stephen Buescher, "Electromagnetic Effects on Biological Cells," Conference Digest of the 25th International Conference on Infrared and Millimeter Waves, September 12–15, 2000, Beijing, China, W-G1, p. 191.

6. Invited: K.H. Schoenbach, R.H. Stark, J. Deng, R. El-Sayed Aly, S.J. Beebe, and E.S. Buescher, "Biological/Medical Pulsed Electric Field Treatments," Conf. Record, 2000 Twenty-Fourth Intern. Power Modulator Symposium, June 2000, Norfolk, VA, p. 42.

7. J. Deng, R.H. Stark, and K.H. Schoenbach, "A Nanosecond Pulse Generator for Intracellular Electromanipulation," Conf. Record, 2000 Twenty-Fourth Intern. Power Modulator Symposium, June 2000, Norfolk, VA, p. 47.

8. Karl H. Schoenbach, Robert J. Barker, and Shenggang Liu, "Nonthermal Medical/Biological Treatments Using Electromagnetic Fields and Ionized Gases," Proc. 12th IEEE Intern. Pulsed Power Conf., Monterey, CA, June 1999, C. Stallings and H. Kirbie, eds., p. 497.

9. Karl H. Schoenbach, "Deactivation of Microbial Pathogens with Intense, Ultrashort Electrical Pulses and High Power Microwave Radiation," Proc. 1998 ERDEC Scientific Conf. on Chemical and Biological Defense Research, Aberdeen Proving Ground, MD, November 1998, p. 583.

C. Published Abstracts

1. Invited: SJ Beebe, PM Fox, M Gellerman, LJ Rec, LK Willis, PS Hair, ES Buescher, J Deng, RH Stark, and KH Schoenbach," High Intensity Pulsed Electric Fields in the Nanosecond Range Bypass the Cell Membrane and Target Subcellular Structures in Human Cells," Abstract Records, SPRBM Meeting, Charleston SC, January 2001.

2. Invited: E. Stephen Buescher, Pamela S. Hair, Stephen J. Beebe, and Karl H. Schoenbach, "Nanosecond Pulsed Electric Field (nsPEF) Applications to Human Cells Results in Selective Intracellular Membrane Disruption," Symposium Abstracts, ElectroMed2001, Portsmouth, VA, May 2001, p. 20.

3. Invited: S.J. Beebe, P.M. Fox, L.J. Gellerman, E. Hall, L.K. Willis, P.S. Hair, E.S. Buescher, J. Deng, R.H. Stark, and K.H. Schoenbach, "High Intensity, Nanosecond Pulsed Electric Fields (nsPEF) Induce Apoptosis in Vitro and in Vivo and Inhibit Tumor Growth in Vivo," Symposium Abstracts, ElectroMed2001, Portsmouth, VA, May 2001, p. 21.

4. Invited: Q.Hu, R.P. Joshi, and K.H. Schoenbach, "Modeling of Biological Cells Subjected to High Intensity Electrical Pulses," Symposium Abstracts, ElectroMed2001, Portsmouth, VA, May 2001, p. 15.

5. Guofen Yu, Elizabeth Coln, Karl H. Schoenbach, Merican Gellerman, Paula Fox, Laura Rec, and Stepehen Beebe, "A Study on Biological Effects of Millimeter Waves," Symposium Abstracts, ElectroMed2001, Portsmouth, VA, May 2001, p. 53.

6. A. Abou-Ghazala, Sunao Katsuki, K.H. Schoenbach, F.C. Dobbs, and K.R. Moreira, "Bacterial Decontamination of Water by Means of Pulsed Corona Discharges," Symposium Abstracts, ElectroMed2001, Portsmouth, VA, May 2001, p. 75.

7. R.P. Aly, R.P. Joshi, R.H. Stark, K.H. Schoenbach, and Stephen J. Beebe, "Repair Time of Bacteria after Pulsed Electric Field Application," Symposium Abstracts, ElectroMed2001, Portsmouth, VA, May 2001, p. 79.

8. S.J. Beebe, P.M. Fox, L.J. Rec, M. Gellerman, E. Hall, L.K. Willis, P.S. Hair, E.S. Buescher, J. Deng, R.H. Stark, and K.H. Schoenbach, "High Intensity, Nanosecond Pulsed Electric Fields (nsPEF) Induce Apoptosis In Vitro And In Vivo And Inhibit Tumor Growth In Vivo," Symposium Abstracts, ElectroMed2001, Portsmouth, VA, May 2001, p. 86.

9. Jingdong Deng, Karl H. Schoenbach, Stephen J. Beebe, and E. Stephen Buescher, "Effects of Microsecond and of Submicrosecond Electrical Pulses on Biological Cells," Symposium Abstracts, ElectroMed2001, Portsmouth, VA, May 2001, p. 95.

10. Michael W. Stacey, E. Steven Buescher, Pamela Hair, Stephen J. Beebe, and Karl H. Schoenbach, "DNA Damage Visualized by Comet Assay Following Nanosecond Pulsed Electric Field Applications to Eosinophils," Symposium Abstracts, ElectroMed2001, Portsmouth, VA, May 2001, p. 113.

11. L.K. Willis, M. Gellerman, E. Hall, P.M. Fox, S.J. Beebe, J. Deng, R.H. Stark, and K.H. Schoenbach, "Nanosecond Pulsed Electric Fields Induce Apoptosis in 3T3-L1 Cells: A Comparison of Apoptosis Induction by Fas," Symposium Abstracts, ElectroMed2001, Portsmouth, VA, May 2001, p. 117.

12. Karl H. Schoenbach, Robert H. Stark, Stephen Beebe, and Stephen Buescher, "Bioelectrics – New Applications for Pulsed Power Technology," Conference Record – Abstracts PPPS2001, Las Vegas, NV, June 2001, p. 56.

13. Stephen J. Beebe, E. Stephen Buescher, Robert H. Stark, and Karl H. Schoenbach, "Nanosecond Pulsed Electric Field (nsPEF) Application Effect on Human Cells: Intracellular Membrane Disruption and Apoptosis Induction," Conference Record – Abstracts PPPS2001, Las Vegas, NV, June 2001, O2A2-O2A3, p. 251.

14. A. Abou-Ghazala, K.H. Schoenbach, F.C. Dobbs, and K.R. Moreira, "Bacterial Decontamination of Water by Means of Pulsed Corona Discharges," Conference Record – Abstracts PPPS2001, Las Vegas, NV, June 2001, P2H06, p. 308.

15. R.E. Aly, R.P. Joshi, R.H. Stark, and K.H. Schoenbach, "Repair Time of Bacteria After Pulsed Electric Field Application," Conference Record – Abstracts PPPS2001, Las Vegas, NV, June 2001, P2H09, p. 310.

16. J. Deng, R.H. Stark, and K.H. Schoenbach, "Development of Compact, Nanosecond, High Voltage Pulse Generators for Biological Studies," Conference Record – Abstracts PPPS2001, Las Vegas, NV, June 2001, P3K08, p. 447.

17. Robert J. Barker and Karl H. Schoenbach, "Plasmas and Pulsed Power for Biomedical Applications," Conf. Record, IEEE Intern. Conf. Plasma Science, New Orleans, 2000, p. 80.

18. K.H. Schoenbach, S. Beebe, and S. Buescher, "Biological Effects of High Power, Microsecond and Submicrosecond Electrical Pulses," Symposium Record, First International Symposium on Nonthermal Medical/Biological Treatments Using Electromagnetic Fields and Ionized Gases, Norfolk, VA, April 1999, p.35.

D. Invited Talks

1. Karl H. Schoenbach, "Nonthermal Pulsed Electric Field Effects on Biological Cells," Seminar, Stevens Institute of Technology, Hoboken, NJ, October 26, 2001.

2. Karl H. Schoenbach, "Bioelectrics – New Applications for Pulsed Power Technology," Seminar, Kumamoto University and Chamber of Commerce at City of Kumamoto, Japan, July 26, 2001.

3. Plenary Talk: Karl H. Schoenbach "Bioelectrics – New Applications for Pulsed Power Technology," PPPS2001, Las Vegas, NV, June 26, 2001.

4. Stephen J. Beebe, "Nanosecond Pulsed Electric Field (nsPEF) Application Effect on Human Cells: Intracellular Membrane Disruption and Apoptosis Induction," PPPS2001, Las Vegas, NV, June 26, 2001.

5. E. Stephen Buescher, "Nanosecond Pulsed Electric Field (nsPEF) Applications to Human Cells Results in Selective Intracellular Membrane Disruption," ElectroMed2001, Portsmouth, VA, May 21, 2001.

6. S.J. Beebe, "High Intensity, Nanosecond Pulsed Electric Fields (nsPEF) Induce Apoptosis in Vitro and in Vivo and Inhibit Tumor Growth in Vivo," ElectroMed2001, Portsmouth, VA, May 21, 2001.

7. R.P. Joshi, "Modeling of Biological Cells Subjected to High Intensity Electrical Pulses," ElectroMed2001, Portsmouth, VA, May 21, 2001.

8. Karl H. Schoenbach, "Bioelectrics – New Applications for Pulsed Electrical Power Technology," IEEE, Hampton Roads Section, Newport News, VA, March 15, 2001.

9. S.J. Beebe, "High Intensity Pulsed Electric Fields in the Nanosecond Range Bypass the Cell Membrane and Target Subcellular Structures in Human Cells," SPRBM Meeting, Charleston SC, January 2001.

10. Karl H. Schoenbach, "Medical and Biological Applications of Pulsed Electric Fields," Graduate Seminar, Electrical and Computer Engineering Department, Old Dominion University, Norfolk, VA, November 17, 2000.

11. Karl H. Schoenbach, "Electromagnetic Effects on Biological Cells," University of Electronic Science and Technology of China, Chengdu, China, September 19, 2000.

12. Karl H. Schoenbach, "Electromagnetic Effect on Biological Cells," 25th International Conference on Infrared and Millimeter Waves, September 12, Beijing, China.

13. Karl H. Schoenbach, "Pulse Power Requirements for Biological/Medical Pulsed Electric Field Treatments," Twenty-Fourth International Power Modulator Symposium, Norfolk, VA, June 27, 2000.

14. Plenary Talk: Robert J. Barker, "Plasmas and Pulsed Power for Biomedical Applications," Plenary Talk, IEEE Intern. Conf. Plasma Science, New Orleans, June 4, 2000.

15. Keynote Address: Karl H. Schoenbach, "Medical and Biological Applications of Pulsed Electric Fields," 2000 High Voltage Workshop, Newport Beach, CA, April 10, 2000.

16. K.H. Schoenbach, "Biological Effects of High Power, Microsecond and Submicrosecond Electrical Pulses," Symposium Record, First International Symposium on Nonthermal Medical/Biological Treatments Using Electromagnetic Fields and Ionized Gases, Norfolk, VA, April 13, 1999.

E. Patent

Method and Apparatus for Intracellular Electromanipulation
Patent No: 6,326,177
Patent Date: December 4, 2001
Inventors: Karl H. Schoenbach, Stephen J. Beebe, and E. Stephen Buescher

Degrees:

Ramy El-Sayed Aly, "The Effect of Multiple, Electrical Pulses on Bacteria," M.S. Thesis, August 2001.

Elizabeth A. Coln "A Study of Radiofrequency Effects on Gene Expression", M.S. Thesis, December 10, 2001.

Jingdong Deng, "Effect of Intense, Submicrosecond Electrical Pulses on Mammalian Cells," PhD Dissertation
Mr. Deng has passed the PhD candidacy exam, but decided to leave PhD program for personal reasons.

Journal Publications

A-1

Simulations of Electroporation Dynamics and Shape Deformations in Biological Cells Subjected to High Voltage Pulses

R. P. Joshi, Q. Hu, and K. H. Schoenbach

Dept. of Electrical & Computer Engineering, Old Dominion University, Norfolk, VA 23529-0246.

S. J. Beebe

Departments of Pediatrics and Physiology, Eastern Virginia Medical School, Norfolk, VA 23501.

The temporal dynamics of electroporation of cells subjected to ultrashort voltage pulses are studied based on a coupled scheme involving the Laplace, Nernst-Plank, and Smoluchowski equations. It is shown that a finite time delay exists in pore formation, and leads to a transient overshoot of the transmembrane potential V_{mem} beyond 1.0 Volt. Pore resealing is shown to consist of an initial fast process, a 10^{-4} second delay, followed by a much slower closing at a time constant of about 10^{-1} second. This establishes a time-window for effective killing by a second pulse. The results are amply supported by our experimental data for *E.-coli* cells, and the time constant also matches experiments. An electro-mechanical analysis for analyzing cell shape changes is also presented. Our calculations show that at large fields, the spherical cell geometry can be significantly modified, and even ellipsoidal forms would be inappropriate to describe the deformation. Values of surface forces obtained are in very good agreement with the 1-10 nN/m range reported for membrane rupture. It is also demonstrated that, at least for the smaller electric fields, both the cellular surface area and volume change roughly in a quadratic manner with electric field. Finally, it is shown that the bending moments are generally quite small and can be neglected for a simpler analysis.

I. INTRODUCTION

Electroporation is a well known physical process in biological cells [1-3]. It involves rapid structural rearrangement of the membrane, in response to an externally applied electric field. The most prominent observable effect is a rapid increase in the electrical conductivity by several orders of magnitude [4]. This is attributed to the formation of aqueous pathways, or pores, in the lipid bilayer of the membrane. The opening of such channels (or more appropriately, transient aqueous pores) enables the transport of ions and water-soluble species both into and out of individual cells. Electroporation can, therefore, be used to initiate large molecular fluxes for purposes of introducing genetic material into cells, manipulation of cells and tissues, and other applications in biotechnology [5-9].

Electroporation has also been linked to the non-thermal killing of micro-organisms subjected to strong electric fields [10]. For this reason, it offers great potential for de-contamination and the elimination of harmful micro-organisms and bio-hazards. Traditionally, most electroporation studies have focused on relatively low external voltages applied over extended time periods ranging from several tens of microseconds to milliseconds [11]. Recently, work has focused on the use of much shorter, high-voltage pulses with electric fields as high as 100 kV/cm [12-15] and durations well below the micro-second range. There appear to be several fundamental advantages in using short electric pulses for cellular manipulation. First, negligible thermal heating of the biological matter can be expected to occur due to the short time duration. Much lower energies are required for pulsed inputs, and yet large values of the electric fields and peak powers can be obtained [16]. Also, pulsed fields afford a way by which the time scales can easily be manipulated.

Electroporation dynamics were first treated based on the Smoluchowski equation by

from an external step voltage are computed based on the current continuity and Laplace equations. Current flows are computed corresponding to the electric fields at each time step from a continuum Nernst-Planck type model that includes diffusion [27, 28, 32]. In keeping with the literature [33-35], it is assumed here that two types of pores, hydrophilic and hydrophobic, exist. Each of the two pore types were characterized by a "formation energy" function, E(r) dependent on the pore radius "r" . The following pore energy function was chosen in keeping with the published and accepted model [4, 27, 36, 37].

$$E(r,t) = 2\,\pi h r\,\sigma(\infty)\,[\,I_1(r/r_0) / I_0(r/r_0)\,] \;-\pi\,a_p\,V^2\,r^2 \quad , \text{ and} \qquad (1a)$$

$$E(r,t) = 2\,\pi\gamma r - [\,{}_0\!\int^{r} 2\pi\Gamma(r^*)r^*\,dr^*\,] \;+\; (C/r)^2 \;-\pi\,a_p\,V^2\,r^2 \quad , \qquad (1b)$$

for hydrophobic and hydrophilic pores, respectively. In the above, I_1 and I_0 are the modified Bessel functions of the zeroth and first order, respectively, h is the membrane thickness, $\sigma(\infty)$ is a constant equal to 5×10^{-2} N m^{-1} , while r_0 represents a characteristic length scale over which the properties of water change between the interface and the bulk, taken to equal 1 nm. A simple heuristic model has recently been used [38] for $\Gamma(r)$ in which $\Gamma(r) = \Gamma_0\,[1 - r^2/r_\infty^2]$. This is based on the idea that tension is proportional to the membrane area, at least to first order. The primary effect of such variations in Γ, would be the creation of an additional local minima in the pore energy function. From the standpoint of electroporation, this means that instead of expanding indefinitely beyond the unstable maxima, the pores ultimately stabilize at a high radial value. The variable tension concept had been proposed by Winterhalter and Helfrich [39], as well.

Typical values for the various parameters are given in Table I. The value of "C" in (1b) was

chosen to be 9.67x 10^{-15} $J^{1/4}$ m in keeping with the reports by Neu et al. [34] as it yields values close to those measured by Glasser et al. [27]. The coefficient a_p is a property of the membrane and its aqueous environment. In the simplest continuum approximation [27], and expressed in terms of the membrane thickness "h" and the permittivities "ε_w" and "ε_m" of water and the membrane, respectively, as : $a_p = (\varepsilon_w - \varepsilon_m)/[2h]$. The energy function E(r) determines the "drift flux" for pores in r-space and therefore, governs the growth or contraction of pores at any given radius "r". In general, the presence of a membrane voltage reduces the maxima, and can even quell the energy barrier completely beyond a critical voltage value. For transient voltage pulses, stability would depend on the ability of pores to drift past the barrier *maximas within the duration of the applied voltage pulse.* It was assumed here that all pores are initially created as hydrophobic (i.e. non-conducting) at a rate S(r) per unit area of the membrane at a rate given by :

$$S(r) = \{(v_c\, h) / (k_B\, T)\}\ [dE(r)/dr] \exp[-E(r)/(k_B T)]\, dr\, dt\,, \quad (2)$$

where v_c is an attempt rate density [36], E(r) the energy for hydrophobic pores, T the operating temperature, and k_B the Boltzmann constant. If a non-conducting pore is created with a radius $r > r^*$ (= 0.5 nm), it spontaneously changes its configuration and transforms into a conducting, hydrophilic pore. All conducting pores then survive as long as their radii remains larger than r^*. Destruction of a conducting pore occurs only if it drifts or diffuses in r-space to a value below r^*. Due to the exponential term in (2), most pores are created with very small radii.

The Smoluchowski equation that governs the pore dynamics is given in terms of the pore density distribution function n(r,t) as :

$$\partial n(r,t)/\partial t + \{D/[k_B T]\} [\partial\{E(r) n(r,t)\}/\partial r] - D [\partial^2 n(r,t)/\partial r^2] = S(r), \quad (3)$$

where S(r) is the source term as given in (2), while D is the pore diffusion constant given in Table I. The process of diffusion represents a "random walk" of the pore radius in "r-space". Numerical simulations of the dynamic pore distribution were carried out based on a time-domain, finite-difference discretization of the governing Smoluchowski equation. An upperbound, r_{max}, of 2000 Å was set on the pore radius, and this entire r-space was uniformly divided into 5000 segments. A "reflecting boundary" was assigned at $r = r_{max}$, which was implemented by setting the pore flux to zero at $r = r_{max}$. Mathematically, this amounts to a Neumenn condition: $[dn(r,t)/dr]|_{r = rmax} = -[dE(r,t)/dr] [n/(k_B T)]_{r = rmax}$. At the other end, absorbing boundary conditions were implemented by setting $n(0,t) = 0$. The time step $dt = 10^{-11}$ second was chosen as it is to be much smaller than the fluctuation rate v_d representing the fastest time constant in the system [27].

The schematic shown in Fig. 1 was used to represent a cell suspended in a medium, and the relevant equations applied to this geometry. The inner spherical region was taken to have a radius "a" and conductivity σ_{in}. The applied electric field $F_0(t)$ was taken to be along the z-axis. The cellular membrane was assigned a thickness "b – a", while the outer region denoting the suspension was assigned a conductivity σ_{out}. Due to axial-symmetry, the potentials satisfying the Laplace equation, could be expressed as:

$$U_{in}(r,t) = A_0(t) P_0 + A_1(t) r P_1 + A_2(t) r^2 P_2 + \ldots = \Sigma_{j=0,\infty} A_j(t) r^j P_j, \quad (3a)$$

$$U_{mem}\ (r,t) = \Sigma_{j=0,\infty} [\ B_j\ (t)\ r^j\ P_j\ + C_j\ (t)\ P_j / r^{j+1}\ ]\ , \qquad (3b)$$

$$\text{and}\ \ U_{out}\ (r,t) = -\ F_0\ (t)\ r\ P_1\ + \Sigma_{j=0,\infty}\ D_j\ (t)\ P_j / r^{j+1}\ , \qquad (3c)$$

where U_{in} (r,t), U_{mem} (r,t), and U_{out} (r,t) are the potentials at the inner-, the membrane- and outer-regions, P_j is the j^{th} order Legendre polynomial, and F_0 (t) the externally applied electric field. Also, A_j (t), B_j (t), C_j(t), and D_j (t) denote the coefficients of the Legendre series expansions that can be determined by applying matching boundary conditions at the interfaces of the three regions. Here, the Laplace instead of Poisson's equation has been used on the assumption that charge inequalities arising from ionic transport can be ignored on the short time scales. Invoking continuity in potential and current density then leads to the following expression for the membrane potential V_{mem} (t) :

$$V_{mem}\ (t) = A(t)\cos(\theta)\ [\ \{b-b^3/a^2\}\ \{1+2\sigma_{out}/\sigma_{in}\}/\ \{(b/a)^3 + 2\sigma_{out}/\sigma_{in}\ \} - (b-a)\ ]$$

$$+\ 3\cos(\theta)\ F_0\ (t)\ (\sigma_{out}/\sigma_{in})\ /\ \{(b/a)^3 + 2\sigma_{out}/\sigma_{in}\ \}\ , \qquad (4a)$$

where θ is the angle with respect to the z-axis (and hence, the applied electric field direction), and A(t) satisfies the following ordinary differential equation :

$$C_M\ [dA(t)/dt\ ]\ [\{b-b^3/a^2\}\ \{1+2\sigma_{out}/\sigma_{in}\}/\ \{(b/a)^3 + 2\sigma_{out}/\sigma_{in}\ \} - (b-a)\ ] = -\ J_{mem}\ (t) -$$

$$-\ \sigma_{in}\ [\ \{\ 2(\sigma_{out}/\sigma_{in})\ \{(b/a)^3 - 1\}\ A(t) - 3\ (\sigma_{out}/\sigma_{in})\ F_0\ (t)\ (b/a)^3\ \}\ /\{(b/a)^3 + 2\sigma_{out}/\sigma_{in}\ \}\ ]\ . \qquad (4b)$$

Under steady-state, the transmembrane potential V_{mem} from the above equation simplifies to: - 1.5

a E cos(θ) for b ~ a, in accordance with reports in the literature [40, 41].

The conduction current density J_{mem} (t) needs to be specified in order to solve for the potentials in (4). A one-dimensional approximation of the Nernst-Planck expression for ionic flow is [27,28,32] :

$$I(t) = \pi\sigma R^2 N(t)\{\exp[q V_{mem}(t)/(k_B T)] - 1\}/\{{}_0\int^h \exp[q V_{mem}(t)\{1-x/h\}/(k_B T) + w(x)]\, dx\}\ , \quad (5)$$

where R(t) is the pore radius, σ the conductivity of the aqueous solution that fills the pore, w(x) the energy barrier to ionic flow through the pores, "h" is the membrane thickness, and N(t) the pore density. A simple trapezoidal form for w(x) as given by

$$w(x) = q A/(k_B T)\{x/h_1\} \quad \text{for } 0 \le x \le h_1\ , \quad (6a)$$

$$w(x) = q A/(k_B T) \quad \text{for } h_1 \le x \le h - h_1\ , \quad (6b)$$

$$\text{and,} \quad w(x) = q A/(k_B T)\{(h-x)/h_1\} \quad \text{for } h_1 \le x \le h - h_1\ , \quad (6c)$$

was used for the barrier energy, and will be applied here. In the above, "A" represents the peak barrier height under zero bias. Values of σ and A are known to be roughly 1.3 S/m [42] and 2.5 Volt [27] , respectively. The parameter h_1 is the length near the pore entrance over which the barrier profile would be changing linearly for an unbiased cell roughly equals 0.15 times the membrane thickness. In addition, an ionic component, I_{ion}, which is orders of magnitude lower in strength, has to be included. This ionic current density is [42]: $I_{ion} = 1.9\ (V_{MEM} + .083)$.

It must be pointed out that the above equation (5) is somewhat inaccurate because: (i) The barrier peak "A" is assumed to be independent of the pore radius. An approximate form, which is correct for an infinitely long cylindrical pore geometry, for w(r) has been derived to be [43] : $w(r) = 5 \times 10^{-9} / r$. In our calculations, this w(r) function was explicitly used. (ii) Next, equation (5) treats the pore radius to be a constant. However, not only would the radius of the pores change under transient conditions upon the application of an external voltage waveform, but also the pores would not all be identical in size. The distribution n(r,t) as predicted by the Smoluchowski equation, would impart a heterogeneous spread in the "R" parameter. (iii) Finally, N(t) which is an integral quantity needs to be obtained through a suitable integration of n(r,t) over r-space. Treating N(t) as a fundamental independent variable is incorrect.

In order to correct the above shortcomings of equation (5), the following modified current-voltage (I-V) characteristics were used in this study :

$$I(t)=\pi\sigma_0\int^{\infty} [r^2\, n(r,t)\, \{\exp[qV_{mem}(t)/(k_BT)]-1\}/\{_0\int^{h} \exp[qV_{mem}(t)\{1-x/h\}/(k_BT)+w(r,x)]\, dx\}], \quad (7)$$

which includes an integration in r-space over the time-dependent distribution n(r,t). In the process, the role of inter-cellular variations and the size distribution of pores are both taken into account. A distribution of barrier energies and their fluctuation from site to site is also automatically included in this formulation.

B. Cell Stress and Deformation:

Our basic stress model is based on the classical small deformation theory of thin, elastic

shells [30]. Since the thickness of cell membranes is on the order of 5 μm, compared to their radii of ~1 μm, the shell theory is quite appropriate. We follow the notation of Flugge [30] for the stresses and moments. Thus, N_ϕ denotes the meridional force per length , N_θ the hoop force per length, and $N_{\phi\theta}$ the shear. Also, p_r , p_ϕ and p_θ are the externally imposed stresses (which could include internal osmotic pressure), while r is the distance from the axis of rotation, r_1 the radius of curvature, and r_2 the distance of intersection of the radius of curvature and the axis of revolution. In the present context, p_r , p_ϕ and p_θ will be non-zero due to the Maxwell stress tensor that arises from the external field. From the geometry, $r = r_2 \sin(\phi)$, while the elemental distance "ds" along the meridian is given by: $ds = r_1 \, d\phi$, where ϕ is the angle between a normal to the shell and its axis of revolution. The parameter θ denotes the meridional angle. Finally, M_ϕ , M_θ and $M_{\phi\theta}$ are the bending moments (dimensions of force), while Q_θ and Q_ϕ transverse forces per length that arise from bending theory. At equilibrium, the balance of all forces and moments yields the following six equations:

$$d\{r N_\phi\}/d\phi + r_1 \, d\{N_{\theta\phi}\}/d\theta - r_1 N_\theta \cos(\phi) - r Q_\phi = - r r_1 p_\phi \, , \qquad (8a)$$

$$d\{r N_{\phi\theta}\}/d\phi + r_1 \, d\{N_\theta\}/d\theta + r_1 N_{\theta\phi} \cos(\phi) - r_1 Q_\theta \sin(\phi) = - r r_1 p_\theta \, , \qquad (8b)$$

$$r_1 N_\theta \sin(\phi) + r N_\phi + r_1 \, dQ_\theta/d\theta + d\{r Q_\phi\}/d\phi = r r_1 p_r \, , \qquad (8c)$$

$$d\{r M_\phi\}/d\phi + r_1 \, d\{M_{\theta\phi}\}/d\theta - r_1 M_\theta \cos(\phi) = r r_1 Q_\phi \, , \qquad (8d)$$

$$d\{r M_{\phi\theta}\}/d\phi + r_1 \, d\{M_\theta\}/d\theta + r_1 M_{\theta\phi} \cos(\phi) = r r_1 Q_\theta \, , \qquad (8e)$$

$$M_{\phi\theta} / r_1 - M_{\theta\phi} / r_2 = N_{\phi\theta} - N_{\theta\phi} \, . \qquad (8f)$$

The current problem of interest involves an inherent axial symmetry along the direction of the

applied electric field. For such axisymmetric cases, the derivatives with respect to the angle θ drop out, while the shearing forces $N_{\phi\theta}$ and $N_{\theta\phi}$, the twisting moments $M_{\theta\phi}$ and $M_{\phi\theta}$, and the transverse shear Q_θ all vanish. Also, the load component p_θ is zero. Consequently, the following simpler set of equations result:

$$d\{r\,N_\phi\}/d\phi - r_1\,N_\theta\cos(\phi) - r\,Q_\phi = -r\,r_1\,p_\phi\,, \qquad (9a)$$

$$r_1\,N_\theta\sin(\phi) + r\,N_\phi + d\{r\,Q_\phi\}/d\phi = r\,r_1\,p_r\,, \qquad (9b)$$

$$d\{r\,M_\phi\}/d\phi - r_1\,M_\theta\cos(\phi) = r\,r_1\,Q_\phi\,. \qquad (9c)$$

The above three equations contain five unknowns, and need to be supplemented by the stress-strain relationships. In the elastic regime, the stresses can be related to the displacements v (transverse) and w (normal) in the following manner [30] :

$$N_\phi = (12\,K/t^2)\,[\{dv/d\phi + w\}/r_1 + \nu\,\{v\cos(\phi) + w\sin(\phi)\}/r]\,, \qquad (10a)$$

$$N_\theta = (12\,K/t^2)\,[\{v\cos(\phi) + w\sin(\phi)\}/r + \nu\,\{dv/d\phi + w\}/r_1]\,, \qquad (10b)$$

$$M_\phi = (K/r_1)\,[d(\{dw/d\phi\}/r_1)/d\phi + \nu\,\{d[\cos(\phi)\{dw/d\phi\}/r]/d\phi\}]\,, \qquad (10c)$$

$$M_\theta = (K/r_1)\,[\cos(\phi)\{dw/d\phi\}/r] + \nu\,d(\{dw/d\phi\}/r_1)/d\phi]\,, \qquad (10d)$$

where K is the flexural rigidity (i.e. bending stiffness), ν the Poisson's ratio, t the shell thickness assumed to be a constant, and w and v the displacements due to deformation along the radial and angular directions. Equations (10a)-(10d) involve the displacements w and v that constitute two

additional unknowns of the problem. Thus, the combined set of equations [9(a)-9(c)] and [10(a)-10(d)] yield a system of seven equations for the seven unknowns that can be solved.

C. Electric Field Analysis:

The electric fields that give rise to the Maxwell stress tensor can be computed based on the Legendre polynomial model for the potential distributions. Tedious, but straight forward calculations yield the following expressions for the electric fields $F_r(r)$ and $F_\phi(r)$ just outside the membrane (i.e. at $r = b_+$):

$$F_r(r=b) = [3 F_0 + 2 (C_1/b^3) \{1 + (b/a)^3 T\}] \cos(\phi) \equiv F_r \cos(\phi), \quad (10f)$$

$$F_\phi(r=b) = \{C_1 / b^3\} \sin(\phi) [1 + (b/a)^3 T] \equiv F_\phi \sin(\phi), \quad (10g)$$

$$\text{where, } C_1 = - 3F_0 \varepsilon_{out} / [\{T \varepsilon_{mem} /a^3\} - 2\varepsilon_{mem} /b^3 + \{2\varepsilon_{out} /b^3\}[1 + (b/a)^3 T], \quad (10d)$$

$$\text{and, } T = \{2\varepsilon_{mem} + \varepsilon_{in}\} / \{\varepsilon_{mem} - \varepsilon_{in}\}, \quad (10e)$$

where ε_{in}, ε_{mem} and ε_{out} are the permittivities of the inner, membrane, and outer regions, respectively. For $\varepsilon_{in} = \varepsilon_{mem} = \varepsilon_{out}$, the above equations reduce to: $U(r) = - F_0 r \cos(\phi)$, $F_r(r) = F_0 \cos(\phi)$, and $F_\phi(r) = - F_0 \sin(\phi)$.

The expressions for the surface forces $N_\phi(\phi)$ and $N_\theta(\phi)$ become:

$$N_\phi(\phi) = [F^* / \{r_2 \sin^2(\phi)\}] \{ {}_0\int^{\phi} r_1 r_2 [\cos(2\alpha^*) \cos(\phi^*) \sin(\phi^*) + \sin(2\alpha) \sin^2(\phi^*)] d\phi^* \}, \quad (11a)$$

$$N_\theta(\phi) = r_2 F^* \cos(2\alpha) - [r_2 / r_1] N_\phi, \quad \text{where } \alpha^* = \tan^{-1}[-\tan(\phi^*) F_\phi / F_r]. \quad (11b)$$

III. RESULTS AND DISCUSSION

Self-consistent simulations based on the coupled Laplace-Nernst-Planck-Smoluchowski equations were carried out next to evaluate the temporal response to ultrashort, external electric pulses. A 10 kV/cm rectangular external electric field pulse with a 4×10^{-6} duration was assumed. These parameters were chosen in keeping with actual pulsed field experiments conducted on *E. coli* in our laboratory to facilitate comparisons between theory and experiment. The cell radius was chosen to be 1.0 μm and a membrane thickness of 5 nm which is roughly characteristic of *E. coli.* Fig. 2 shows the dynamic evolution of the pore density. An initial delay of about 5 ns seen in Fig. 2a is due to membrane charging and for V_{mem} to build up to levels at which the pore creation rate becomes substantial. A peak value of about 2×10^{14} m^{-2} is reached after about 20 ns. Subsequently, the pore density shows a slight monotonic decrease over the remaining duration of the 4 μs external pulse. This occurs due to a substantial increase in the membrane conductance and a consequent decay in V_{mem} that controls the pore generation. Details of the time dependent membrane voltage are shown in Fig. 3. The voltage exceeds 1.0 volt at about 15 ns, and reaches a peak value of roughly 1.2 volts. At this point the pore conductance increases to such a degree that the voltage across the membrane capacitance begins to fall. The overall result is a "voltage overshoot" behavior. It agrees well with a previous report on the time dependent behavior of the membrane voltage by Meier et al. [29]. As the external electric field is turned off beyond 4 μs, the transmembrane potential falls dramatically with a time constant in the sub-microsecond range. The fast drop off is the result of a large conductance, and hence, a low internal "RC" time constant. A final steady state value of about –80 mV, equal to the rest potential is finally reached. The corresponding influence on the pore density, as seen from Fig. 2a, is a sharp decrease by about fifty percent following the turn-off. Beyond this, the density continues to decrease, but at a relatively low rate until about 0.1 ms. This

implies that many of the pores tend to remain open, well after the 4 μs pulse is switched off. Hence, a second electric pulse applied within this duration is likely to have a substantial damaging effect. This is borne out in our experimental measurements on *E. coli*, as discussed later. Also, this 0.1 ms time delay corresponds well with an experimental report by Meier et al. [29]. Beyond 0.1 ms, the rate of pore reduction increases. The long time behavior can best be gauged from the semi-logarithmic curve of Fig. 2b. It shows a gradual slowing in the resealing rate. At the 2.4 ms instant, an "effective decay time constant" of 4.5×10^{-3} seconds is computed which leads to a pore resealing time of about 0.12 second. In actuality, though, the duration would be even longer since the decay curve of Fig. 2b exhibits a continuous slowing-down. In any case, the resealing values projected values are in the $10^{-1} - 10^{2}$ second range, in keeping with experimental reports [44, 45].

The dynamical behavior can easily be understood in terms of the formation energy characteristic. Initially, the pores that are not near the local minima, and have values in r-space that are below the barrier. These pores tend to drift and diffuse towards $r \rightarrow 0$, giving rise to a fast decay. However, this leaves behind an ever-increasing higher fraction of larger pores that are near the local minima. Those near the minima move in r-space primarily through diffusion, and hence, the system takes a long time to completely recover to the original steady state.

The effect of including a second electric pulse and the comparative temporal behavior is shown in the curves of Fig. 4. The pore density evolution for single and a dual 4 micro-second, 10 kV/cm rectangular electric pulses is shown. The delay for the two-pulse situation was taken to be 1.2 ms. Despite being outside the 0.1 ms time-window, the application of a second pulse is seen to have effect on the evolution. First, the pore density is increased leading to a short spike. However, this increment is not as large as that produced by the original pulse, since the larger pores remain

open, giving rise to a reasonably large membrane conductivity. Consequently, the membrane potential developed is not as large, and the pore creation rate not boosted as much. A second observation is that a two-step decay is evident following the second pulse, as with the initial pulse. Finally, and most important, the decay time for the two-pulse situation becomes significantly longer beyond 2 ms. This implies that applying two or more pulses can slow resealing considerably. From a practical standpoint, this has applications for drug delivery over prolonged durations.

For a more quantitative evaluation, it is instructive to examine the pore distribution functions at various times. Shown in Fig. 5 are five distribution functions at various times for the single- and dual-pulse scenarios. The highest curve for the dual-pulse situation is at a time instant of 1.205 ms and hence, just after the completion of the second pulse. Though the peak lies at about 1.5 nm, a fairly broad "tail" at larger radii is apparent. At a later time of t=1.23 ms for the dual-pulse situation, the peak is seen to be reduced appreciably and broadened due to diffusion in r-space. Finally, at the longest time of 2.43 ms, the dual-pulse distribution is seen to have become quite negligible for small pore radii, but has a well-defined peak at about 52 nm. This location corresponds to the potential minima for the $r_* = 65$ nm curve. It is thus evident that a well-defined population of relatively large pores remains and is long-lived. For the single-pulse situation, the snapshot distributions at t = 1.2 ms, and 2.4 ms are shown. The distribution is relatively broad at 1.2 ms, and similar to the dual-pulse case, settles down to acquire a local peak at 52 nm. However, the 52 nm peak is smaller, signifying a greater number of pores for the dual-pulse case. Beyond these times, the resealing can be expected to be slow, and pore may remain open for a very long time.

Some experimental findings and measured data for *E. coli* cells subjected to short electric pulses are presented. The results, plotted in Fig. 6, show the measured viability of *E. coli* subjected

to two 4 μs, 13 kV/cm electric pulses with variable delays. The delay between the first and second pulses ranged from 5 μs to 50 seconds. A sudden jump in the viability is seen in going from a 0.1 ms delay to a 1.0 ms delay for the second pulse. This is in keeping with the simulation result of Fig. 3a that shows a 0.1 ms time-window during which the pores are mostly open, making the system most vulnerable to destruction by a second electric pulse. Second, there is no significant change in the experimental viability in going from a 1.0 ms delay to a 1 second delay. This again is in keeping with the prediction of a slow pore decay, and the calculated lower bound of about 0.12 seconds. Finally, the absence of complete saturation in the viability even for delay times as long as 50 seconds, suggests that the pore are relatively long lived in accordance with previous reports [44, 45].

Numerical calculations of the cell deformations based on the electro-mechanical stresses given in the previous section were performed to determine the electric field effect on cellular shape. Results for an initial spherical cell of thickness 2 nm having a 1 μm radius (typical of *E. coli* cells, for example) in response to various electric field values are given in Fig. 7. Field magnitudes ranging from 0 – 70 kV/cm were used. The Poisson's ratio ν was taken to be 0.2. The steady-state deformed cell shapes for positive z- and y-variables in the x=0 plane, are shown in Fig. 7. Due to the inherent symmetry of the problem, only the first quadrant is specified for simplicity. The shape changes from a perfect circle for E = 0 V/cm, to ellipsoidal with increasing eccentricity at higher fields. The corresponding forces per length $N_\phi(\phi)$ and $N_\theta(\phi)$ are shown in Fig. 8 for fields of 20, 50 and 70 kV/cm. The magnitudes range from 0 to about 25 nN/m. As reported in the literature [38], the typical tension for membrane rupture is in the range 1-10 nN/m. Our results are thus in very good agreement, and show that for applied electric fields of 50 kV/cm and higher, one can expect

membrane rupture simply based on electro-mechanical considerations. The exact value will obviously depend on the rigidity parameter K and the Poisson's ratio ν, but the magnitudes as predicted by this simply analysis should roughly remain valid.

The deformed cell shape, as will now be demonstrated, strongly depends on the cell characteristics. Changes in the rigidity parameter or the membrane thickness alter the force distributions, and hence, affect the overall shape. Calculated results of the deformed geometry for a 1 μm radius starting from an unstressed spherical cell are given in Fig. 9. The membrane thickness ranged from 2-5 nm and various electric fields were used. The curves of Fig. 9 show very clearly that besides applied electric fields, the deformation is controlled by the membrane thickness, and increases with "t". As the thickness changes from 2 nm to 5 nm, the geometry is modified from spherical to ellipsoidal and then begins to assume a "peanut" shape. Based on the trend evident in Fig. 9, one could qualitatively predict an eventual shift towards a "dumbbell" geometry at higher fields, or thicker membranes, or under conditions of smaller rigidity parameter, or with a larger Poisson's ratio. Such calculations for strongly deformed shapes, however, have not been shown here since the perturbative theory used in this analysis could be called into questions for large deformations.

Fig. 10 shows the bending moment M_ϕ (ϕ) and associated transverse force $Q_\phi(\phi)$ for an applied field of 20 kV/cm for an initial 5 nm sphere. As seen from the curve, the magnitude of M_ϕ (ϕ) is negligibly small and has a nearly constant value of about 3×10^{-12} Newtons. The curve for M_θ (ϕ) was nearly identical to that of M_ϕ (ϕ), and so has not been shown separately in the figure. This M_θ (ϕ) ~ M_ϕ (ϕ) condition obtained here is in keeping with a previous result reported by Pamplona and

Calladine [46]. The angular dependence of $Q_\phi(\phi)$ from Fig. 10 is seen to be symmetric about $\phi = 45^\circ$, and also has a relatively small value. Thus, compared to both $N_\phi(\phi)$ and $N_\theta(\phi)$, the variables $Q_\phi(\phi)$, $M_\theta(\phi)$, and $M_\phi(\phi)$ can all be neglected as has been done in the past. Finally, Fig. 11 shows the fractional change in the cellular surface area and volume as a function of the applied electric field. Two points are evident from the results. First, both curves exhibit a rough quadratic behavior. This is in keeping with some recent optical scattering experimental data [47]. The exact magnitudes, however, are subject to the inaccuracies and uncertainty of the material parameters such as the rigidity K and Poisson's ratio ν. Hence, the data of Fig. 11 does not lend itself to a direct comparison with experimental data. However, the general electric-field dependent trend predicted here has been shown to be accurate. A second point about Fig. 11 is that the change in cell volume is larger than the corresponding areal variation. This is to be expected as the volume scales more rapidly than the surface area, at least for the simple ellipsoidal shapes. At higher electric fields beyond the 25 kV/cm value shown in Fig. 11, it is conceivable that the areal variations become larger as the cell changes from an ellipsoidal to a "peanut-geometry".

IV. SUMMARY AND CONCLUSIONS

A self-consistent model analysis of electroporation in biological cells has been carried out based on the coupled Laplace-Nernst-Planck-Smoluchowski equations. The physical processes of pore generation, drift and diffusion in r-space were all comprehensively included. A pore-radius dependent energy barrier to ionic transport, accounted for cellular variations. This enables predictions and qualitative understanding of the cellular response to short, high electric field pulses

by taking account of the growth and resealing dynamics. The electroporation dynamics in the presence of multiple electric pulses and the potential benefits have also been analyzed.

It has been shown that a finite time delay exists in pore formation, and leads to a transient overshoot of the transmembrane potential V_{mem}. The membrane potential itself remains around the 1.0 Volt value that has been reported in the literature. However, the peak can exceed this value on a transient basis. It has also been demonstrated that pore resealing is a multi-step process. It consists of an initial fast decay, followed by a 10^{-4} second delay, and then a much slower pore-closing with a time constant of about 10^{-1} second. This establishes a 0.1 millisecond (ms) time-window during which the pores are mostly open, and hence, the system is most vulnerable to destruction by a second electric pulse. The existence of such a time window threshold for effective killing by a second pulse is amply supported by our experimental data for *E.-coli* cells involving two pulses with variable delays. A sudden jump in the experimental cell viability has been observed in going from a 0.1 ms delay to a 1.0 ms delay for the second pulse. This is in keeping with our simulation results that show a 0.1 ms time-window during which the pores are mostly open. Second, no significant change in the experimental viability was recorded in going from a 1.0 ms delay to a 1 second delay. This again is in keeping with the prediction of slow pore decay, and the calculated lower bound of about 0.12 seconds. The time constant for the longer process also matches experimental results.

It has also been shown that the pore decay time for the two-pulse situation becomes significantly longer than a single pulse case. This implies that applying two or more pulses can slow resealing considerably. From a practical standpoint, this has applications for drug delivery over prolonged durations, or for controlled manipulation of the pore "open times" via ultrashort, multiple pulses.

In addition, a model analysis of cellular deformation and shape change in response to an applied electric field has also been carried out. A first principles approach based on thin-shell theory was used to evaluate the deformations. This approach utilizing the force and moment equations has the advantage that it can potentially be extended to include dynamical analysis and temporal response. The energy-based schemes would fail in this regard. The present calculations demonstrated the following features: (i) At low values of the applied electric fields (as was commonly the case in the past), the deformed cells can roughly be approximated by an ellipsoidal shape. (ii) However, for much larger field magnitudes, as have recently been used [13-16], the deformations would be fairly significant and the cell geometry would no longer be described accurately by ellipsoidal shapes. For example, at fields on the order of 50 kV/cm, a "peanut-shaped" geometry was shown to result. (iii) The results here also demonstrated that the final shape depends on the membrane thickness. In general, it was argued that with decreasing thickness, deviations from the unstressed shape would be less severe. This has direct implications for cells, tissues and lipid bilayers in which significant molecular re-orientation and re-structuring can occur upon the application of an electric field. (iv) Values of the surface forces obtained in the present calculations were in remarkably good agreement with the 1-10 nN/m range for membrane rupture that has been reported in the literature [38]. This lends validity and credence to the present approach and calculations. (v) It was also shown that, at least for the smaller electric fields, both the cellular surface area and volume would change roughly in a quadratic manner with electric field. (vi) Finally, it was shown that the bending moments are generally quite small and can be neglected for a simpler analysis.

Acknowledgments: The authors would like to thank H. P. Hjalmarson (Sandia National Laboratory) for useful discussions. This work was sponsored in part by the Air Force Office of Scientific Research. Support from Old Dominion University is also gratefully acknowledged.

Table I Parameters Used For The Theoretical Model

PARAMETER	SOURCE	VALUE
$D\ (m^2\ s^{-1})$	Ref. 19	5×10^{-14}
$\gamma\ (J\ m^{-1})$	Ref. 19	1.8×10^{-11}
$\Gamma_0\ (J\ m^{-2})$	Ref. 19	10^{-3}
$C\ (J^{1/4}\ m)$	Ref. 27	9.67×10^{-15}
$K_w\ (F\ m^{-1})$	Ref. 19	$80 \times 8.85 \times 10^{-12}$
$K_m\ (F\ m^{-1})$	Ref. 19	$2 \times 8.85 \times 10^{-12}$
h (m)	Ref. 48	5×10^{-9}
$a_p\ (F\ m^{-2})$	Ref. 27	6.9×10^{-2}
$v_c\ (m^{-3}\ s^{-1})$	Ref. 36	2×10^{38}
$v_d\ (s^{-1})$	Ref. 48	10^{11}
r_o (m)	Ref. 48	1×10^{-9}
$\sigma_0\ (N\ m^{-1})$	Ref. 48	5×10^{-2}
σ_{in} (S/m)	Ref. 15	0.455

σ_{out} (S/m)	Ref. 15	5.0
σ (S/m)	Ref. 15	1.3
A (volt)	Ref. 48	2.5
$n = h_l / h$	Ref. 15	0.15
r_∞ (m)	Ref. 34	65×10^{-9}
K (J)	Ref. 49	5×10^{-20}

FIGURE CAPTIONS

Fig. 1 Schematic of the model used to represent a cell in a suspension for potential calculations.

Fig. 2 Simulation result for the pore density evolution with time in response to a 4 micro-second, 10 kV/cm rectangular electric pulse. (a) Logarithmic scale, and (b) Semi-logarithmic graph.

Fig. 3 Calculated temporal variations of the transmembrane potential in response to a 4 micro-second, 10 kV/cm rectangular electric pulse.

Fig. 4 Simulation result comparing the pore density evolution with one and with two 4 micro-second, 10 kV/cm rectangular electric pulses. The delay for the two-pulse simulation was taken to be 1.2 milliseconds.

Fig. 5 Simulated pore distribution functions at various times for single- and dual-pulse situations.

Fig. 6 Experimental results on the viability of *E. coli* subjected to two 4 μs, 13 kV/cm electric pulses with variable delays. The delay between the first and second pulses ranged from 5 μs to 50 seconds.

Fig. 7 Calculated equilibrium cell shapes along the y-z plane in response to applied electric fields

of 0, 20, 50 and 70 kV/cm.

Fig. 8 Corresponding forces per length N_ϕ (ϕ) and N_θ (ϕ) for applied fields of 0, 20, 50 and 70 kV/cm.

Fig. 9 Deformed cell shape results for various membrane thicknesses and applied fields of 20 kV/cm and 50 kV/cm.

Fig. 10 The bending moment M_ϕ (ϕ) and associated transverse force Q_ϕ(ϕ) for applied field of 20 kV/cm for an initial 5 nm spheroid.

Fig. 11 Calculated variations in the cellular surface area and volume with applied electric field for an initial 1 μm cell radius and 5 nm membrane thickness.

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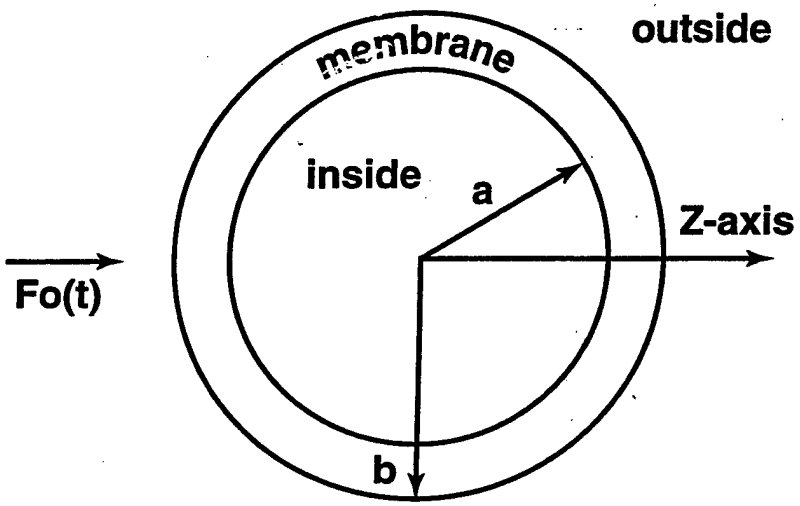
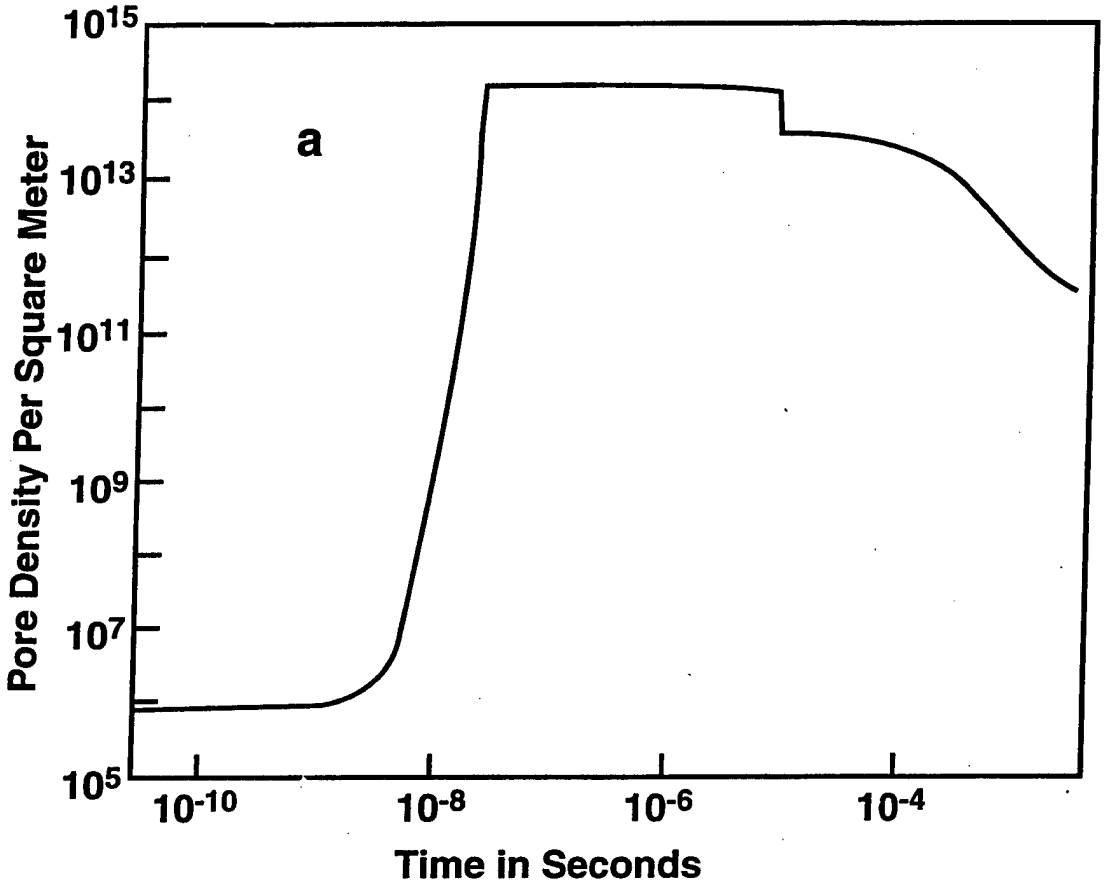

membrane
outside
inside
a
Z-axis
Fo(t)
b


a
Pore Density Per Square Meter
10^{15}
10^{13}
10^{11}
10^{9}
10^{7}
10^{5}
10^{-10}
10^{-8}
10^{-6}
10^{-4}
Time in Seconds

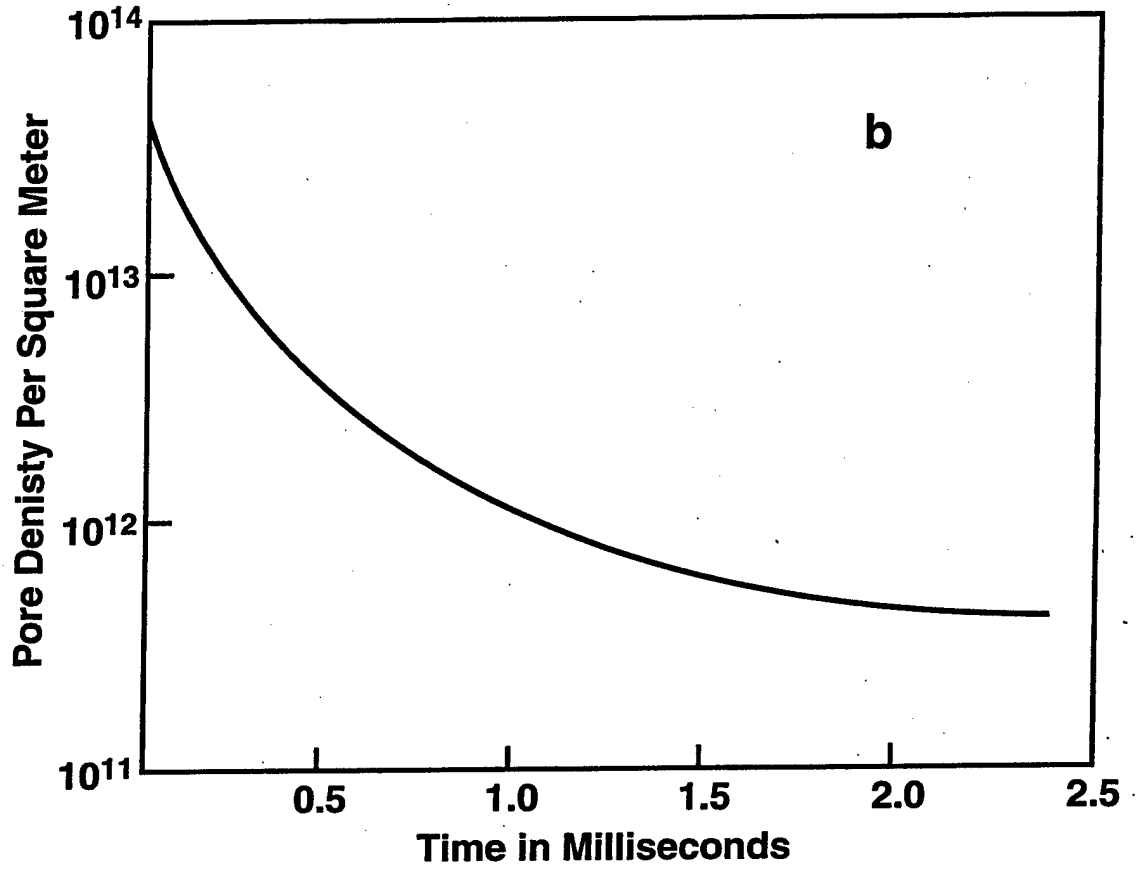
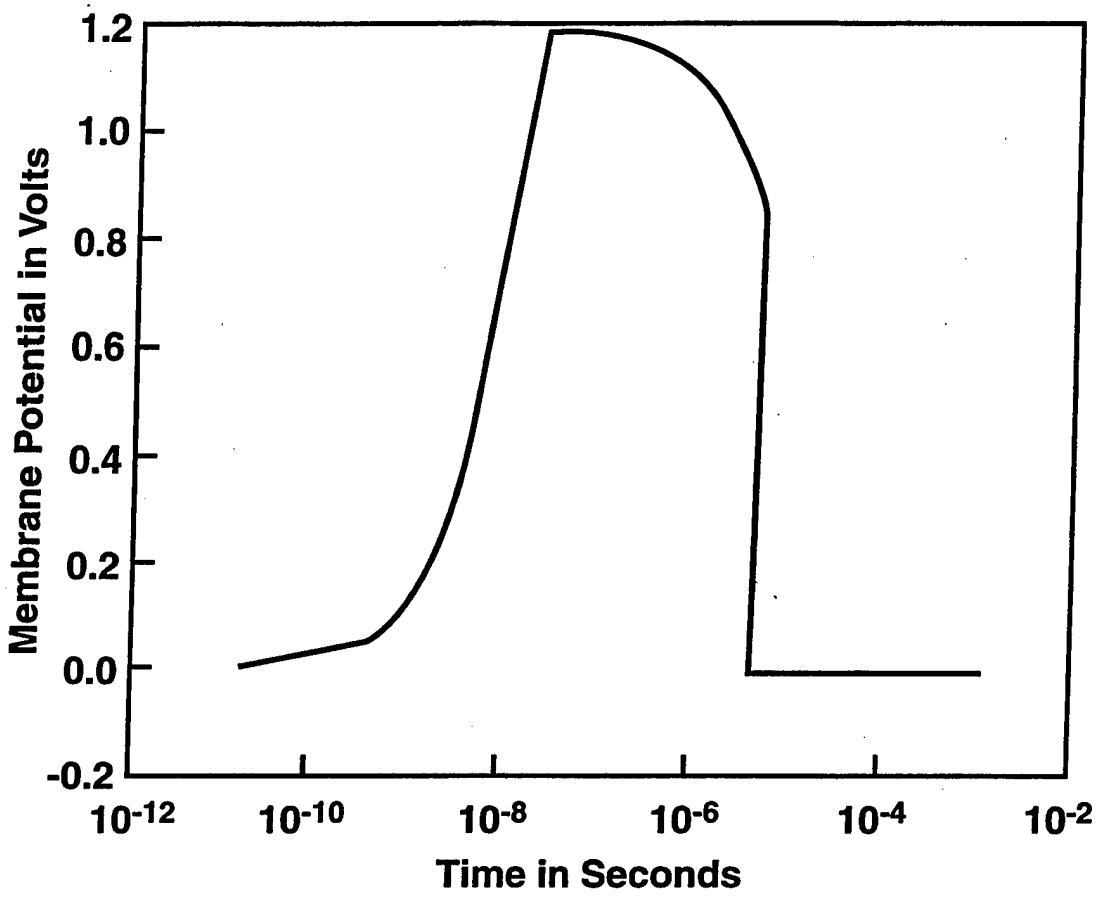


Figure 2b. Jesse et al. IEEE Trans N.S.


1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Membrane Potential in Volts
10-12
10-10
10-8
10-6
10-4
10-2
Time in Seconds

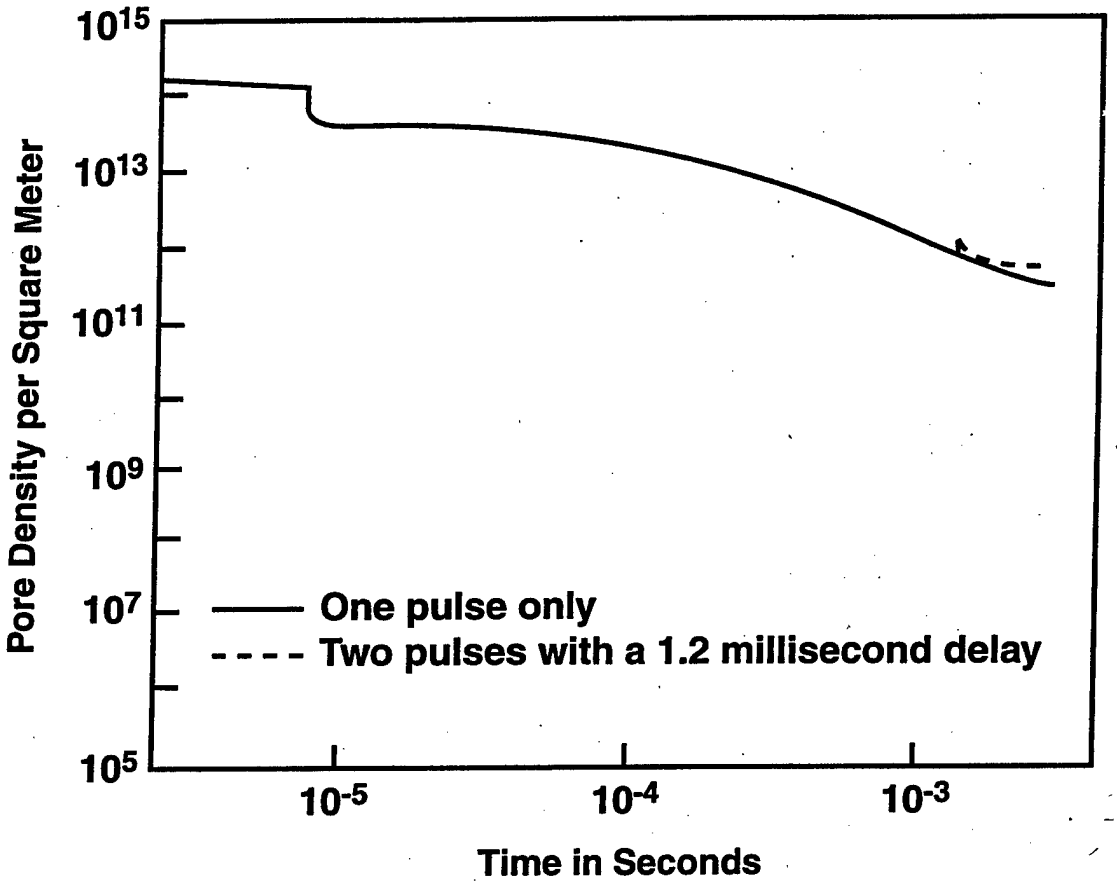

Pore Density per Square Meter
10^15
10^13
10^11
10^9
10^7
10^5
One pulse only
Two pulses with a 1.2 millisecond delay
10^-5
10^-4
10^-3
Time in Seconds

FIG. 4,

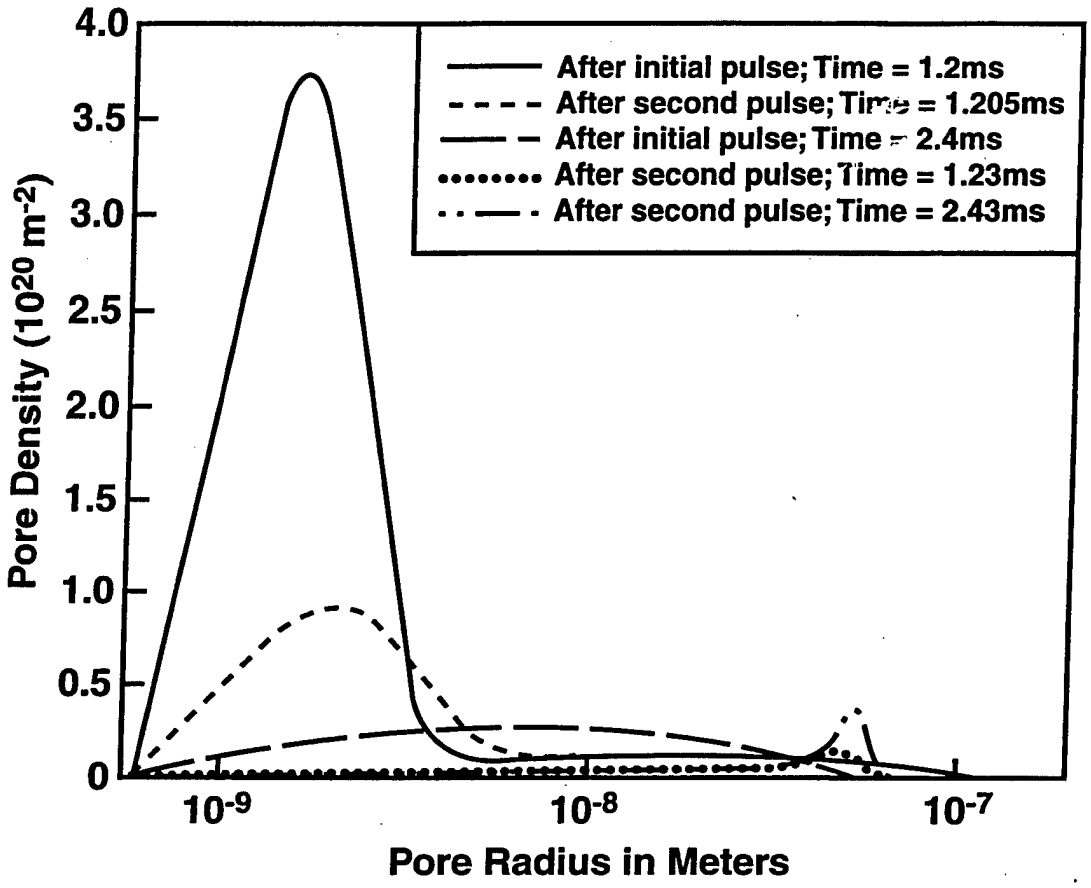

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Pore Density (10^{20} m^{-2})
After initial pulse; Time = 1.2ms
After second pulse; Time = 1.205ms
After initial pulse; Time = 2.4ms
After second pulse; Time = 1.23ms
After second pulse; Time = 2.43ms
10^{-9}
10^{-8}
10^{-7}
Pore Radius in Meters

FIG. 5

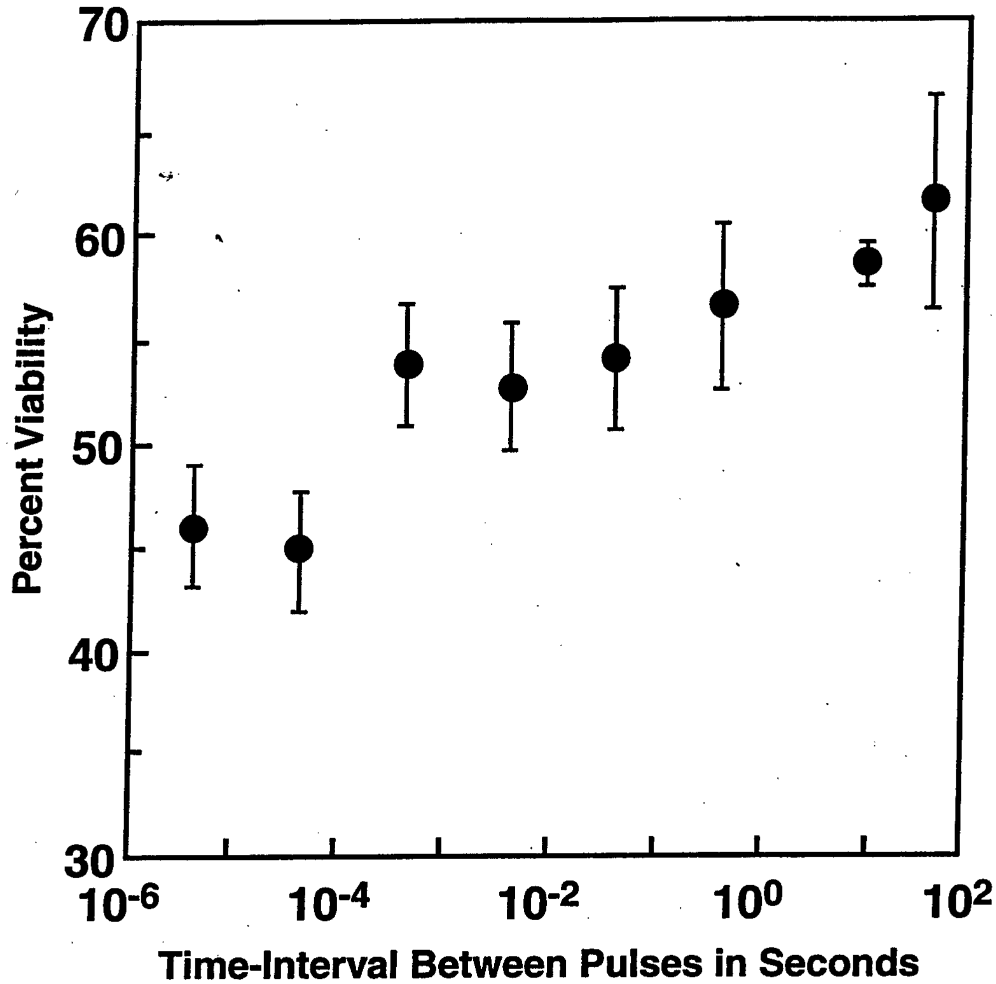
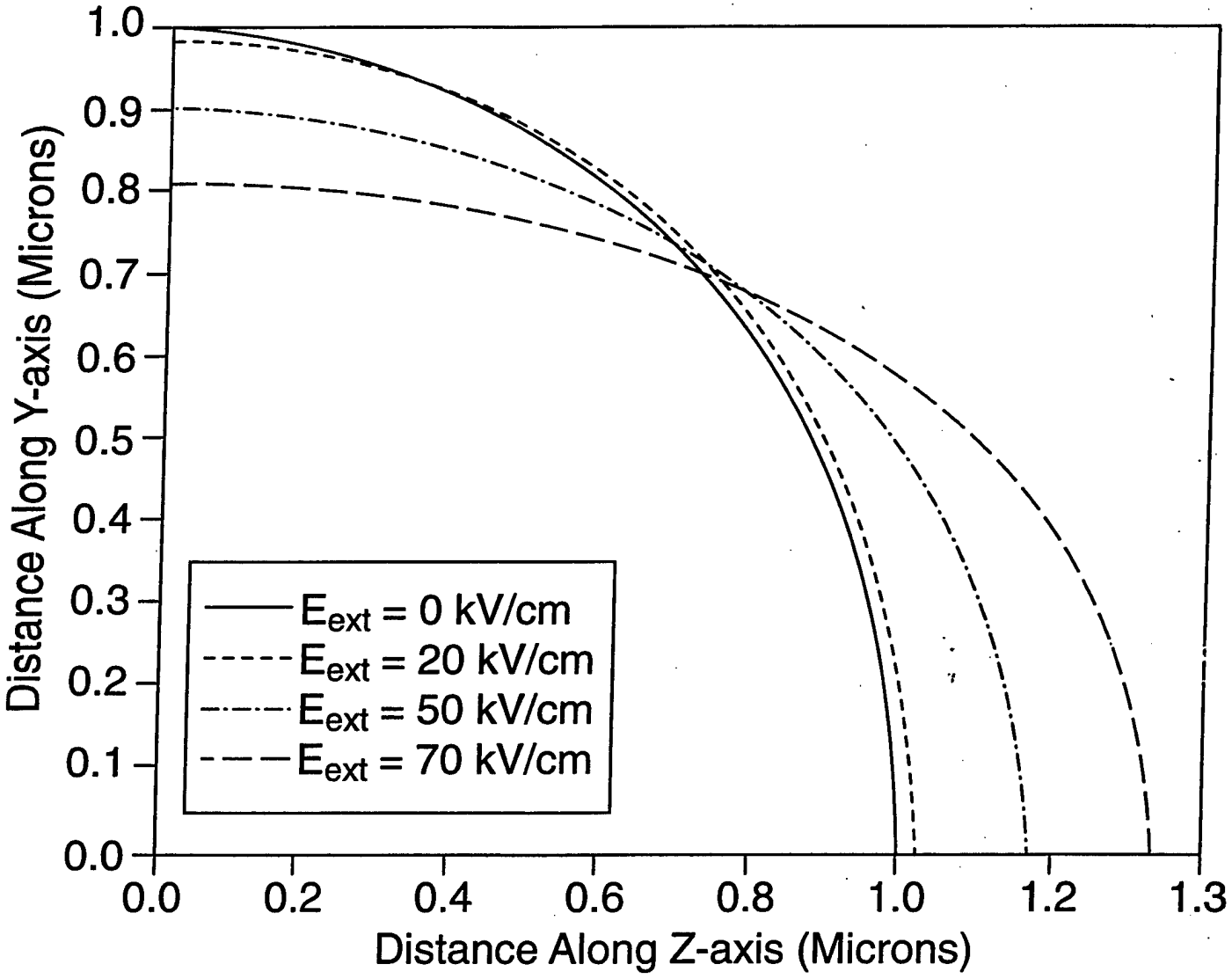
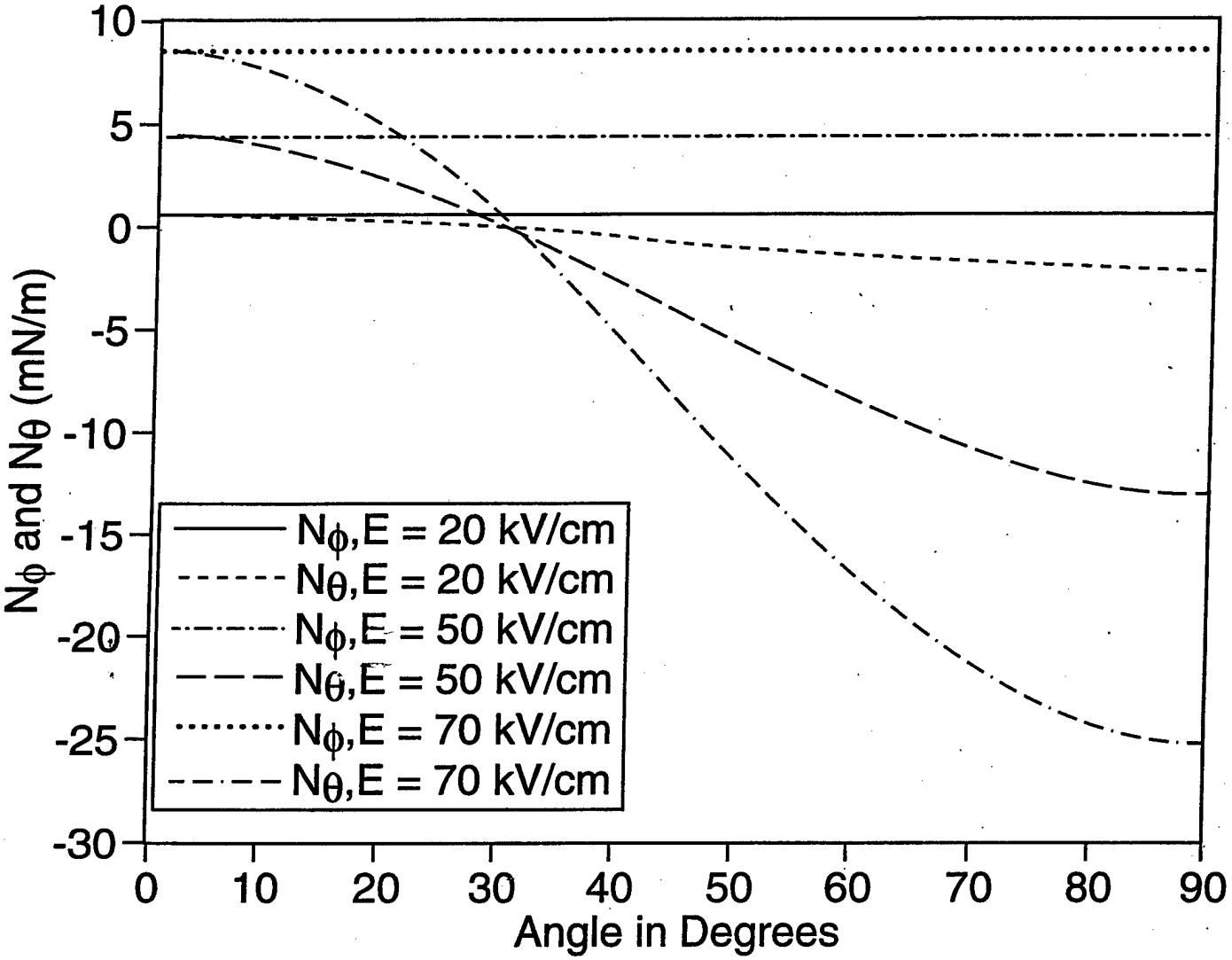
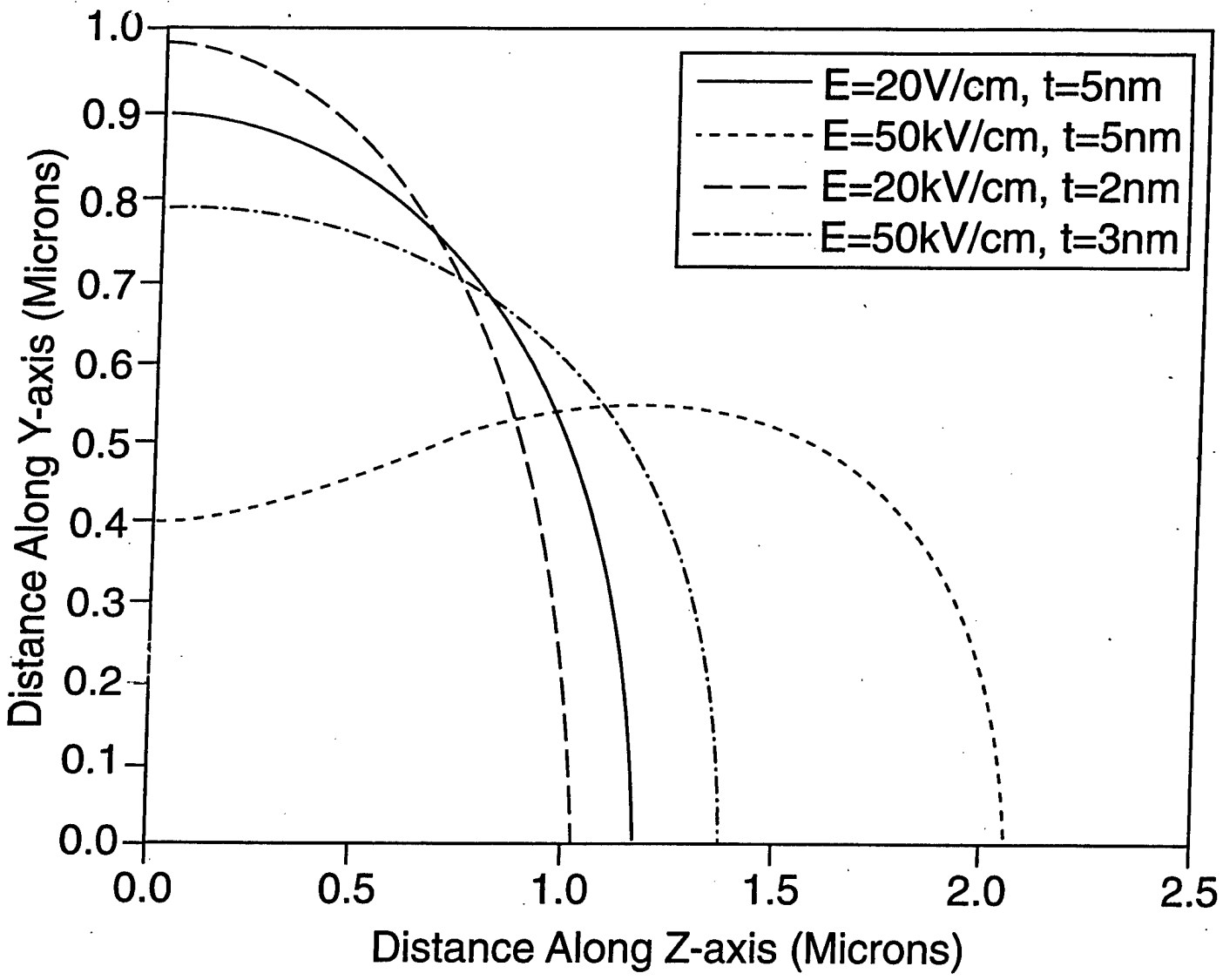
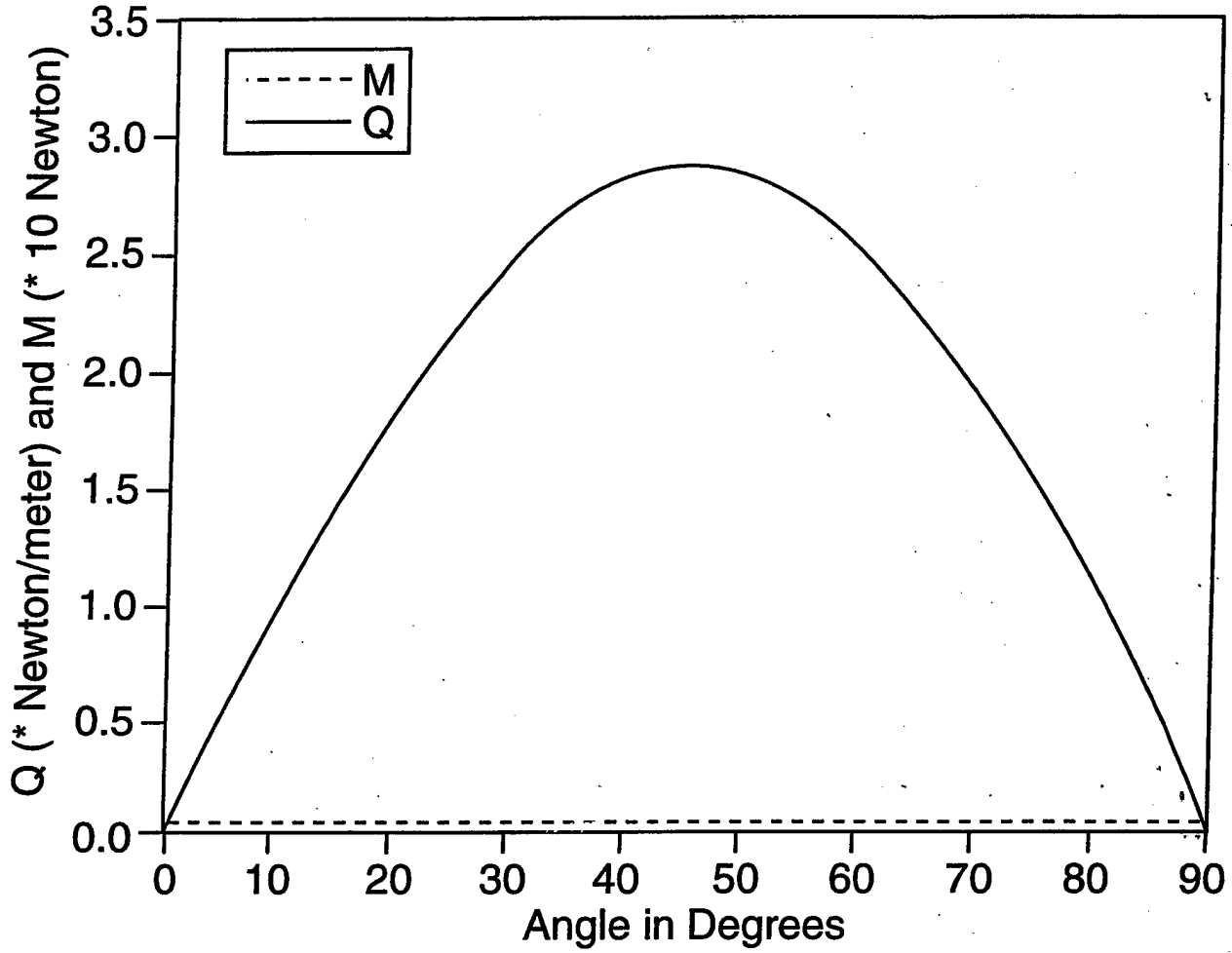


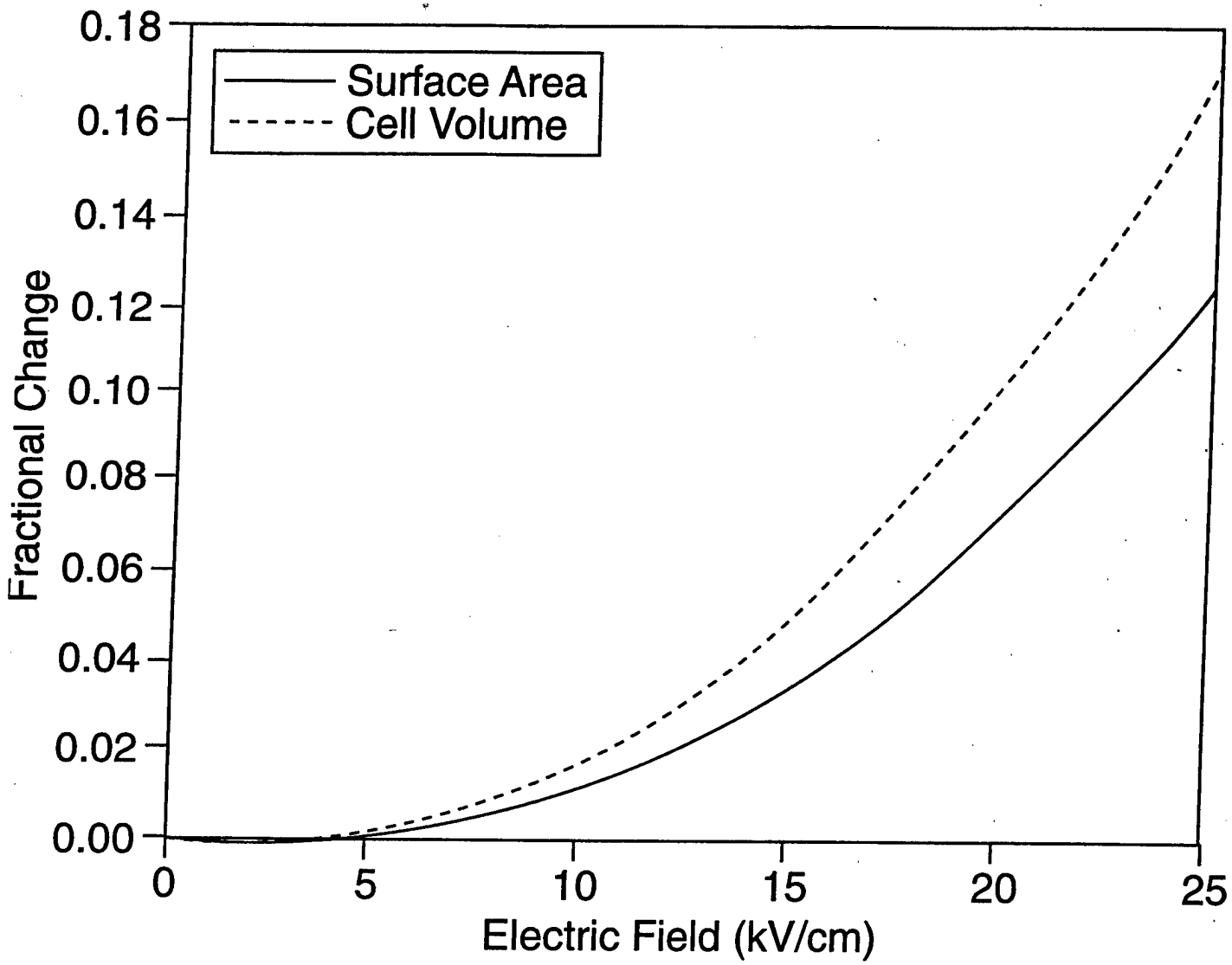
FIG. 6. Joshi et al. [illegible]


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Distance Along Y-axis (Microns)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.3
Distance Along Z-axis (Microns)
E_{ext} = 0 kV/cm
E_{ext} = 20 kV/cm
E_{ext} = 50 kV/cm
E_{ext} = 70 kV/cm


10
5
0
-5
-10
-15
-20
-25
-30
0
10
20
30
40
50
60
70
80
90
Angle in Degrees
Nφ and Nθ (mN/m)
Nφ,E = 20 kV/cm
Nθ,E = 20 kV/cm
Nφ,E = 50 kV/cm
Nθ,E = 50 kV/cm
Nφ,E = 70 kV/cm
Nθ,E = 70 kV/cm


E=20V/cm, t=5nm
E=50kV/cm, t=5nm
E=20kV/cm, t=2nm
E=50kV/cm, t=3nm
Distance Along Y-axis (Microns)
Distance Along Z-axis (Microns)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.5
1.0
1.5
2.0
2.5


M
Q
Q (* Newton/meter) and M (* 10 Newton)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
80
90
Angle in Degrees


Surface Area
Cell Volume
Fractional Change
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
5
10
15
20
25
Electric Field (kV/cm)